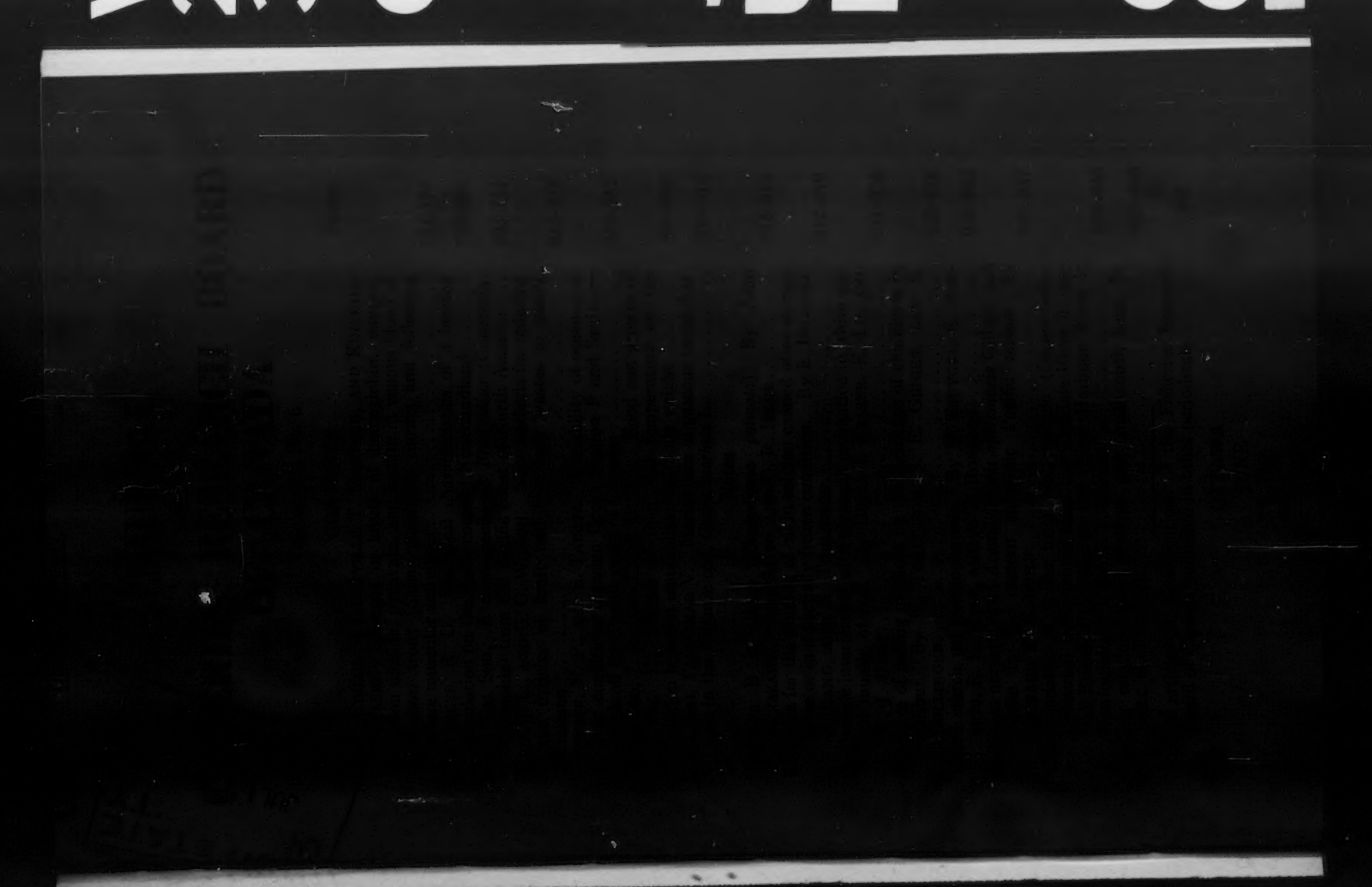

Changes in Levels of Hemoglobin, Oxygen, Carbon Dioxide, Pyruvate, and Lactate in Venous Blood of Rainbow Trout (*Salmo gairdneri*) During and Following Severe Muscular Activity[1,2,3]

By Edgar C. Black, Glenville T. Manning,[4] and Koichiro Hayashi[5]

ABSTRACT

The effects of severe muscular exercise were studied in 1½-year-old rainbow trout (*Salmo gairdneri*). Changes in the oxygen and carbon dioxide content of blood taken from the heart together with alterations in blood levels of pyruvate and lactate were followed in 14 separate conditions. Observations were also made on fish swimming steadily in a rotating annulus.

INTRODUCTION

In man it is known that chemical changes supporting muscular metabolism differ quantitatively for severe activity and moderate activity. When the oxygen supply to active muscles becomes inadequate the muscles derive energy from the anaerobic metabolism of glycogen. These glycogen stores are not very great and soon become severely reduced if not depleted. At the same time lactate accumulates. Similar changes occur in fishes, however, the state of fatigue, as indicated by the presence of large quantities of lactate, persists for several hours in fishes as opposed to several minutes in man.

The nature and extent of these changes in the blood of fishes following severe muscular activity were first studied in Europe by von Buddenbrock (1938) and Secondat and Diaz (1942). They followed the influence of the breakdown products of glycogen upon *p*H, carbonate, and the affinity of hemoglobin for oxygen. They showed that lactic acid accumulated in the blood following struggling or severe exercise. They also showed that the severe acidosis caused a reduction in blood bicarbonate and reduced the oxygen capacity. Black (1957, a, b, c) and Black et al. (1959, 1962) followed changes in the blood of three different salmonids during and following severe muscular activity. They obtained data which substantiated von Buddenbrock and Secondat. However, their studies did not include oxygen, and further observations made on carbon dioxide were studied only with changes in lactate.

In the present paper, blood levels of oxygen, carbon dioxide, pyruvate, and lactate were determined for one and the same sample of blood drawn during and after severe exercise in rainbow trout.

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METHODS AND MATERIALS

Rainbow trout were used for all experiments. The stock was obtained as eggs from Oregon and or California hatcheries and reared in the Summerland trout hatchery, operated by the Fish and Game Branch of the Department of Recreation and Conservation, Government of British Columbia. The fish were 15–17 months old when used. They were fed dry pellet food marketed by J. R. Clark and Co., Utah. Water temperature of the artesian supply ranged from 11 to 12 C.

Severe exercise and recovery as well as moderate exercise were carried out as described in earlier papers by Black (1955, 1957a, b, c). By severe exercise is meant chasing the fish continuously and vigorously for 15 min. By moderate exercise is meant the steady swimming pace adopted by the fish when rotating against the stimuli induced by Fry's rotating annulus.

The method of taking blood samples and methods of analyses were those described in earlier papers (Black, 1955, 1957a, b, c; Black et al., 1959). Oxygen present in the venous blood samples was extracted and measured by the micro-method of Scholander and Roughton (1943).

All determinations were made on blood taken from the same cardiac puncture.

RESULTS AND DISCUSSION

Results are presented in Table I and illustrated in Fig. 1–3.

Body Weights

The body weights of the rainbow trout for all conditions examined averaged 198.61 g and ranged from 102 to 338 g.

Hemoglobin

The average hemoglobin value for the rested condition was 8.01 g/100 ml, a value which is less than 10.2 $\pm$ 0.66 g/100 ml reported by Black et al. (1962). There was a significant increase ($p < 0.05$) to 10.1 g/100 ml following 15 min severe exercise, a value which compares with 13.7 $\pm$ 0.81 g/100 ml reported by Black et al. (1962). The level of hemoglobin varied considerably during the recovery period. At the end of 24 hr of recovery, following 15 min severe exercise, the average value was 9.1%. It is possible that the changes in hemoglobin reflect osmotic shifts of water between muscle and blood. Plasma skimming may also be a factor contributing to the variability of the data.

Oxygen

The oxygen content of the blood taken from the heart is presented for various conditions in Table I and Fig. 1.

The average oxygen content for the unexercised fish was 1.56 vol per cent (i.e. millilitres of oxygen per 100 ml blood) for the range 0.13–3.60 vol

per cent. It is possible that the very low values may have been altered by the immediate past history of the fish before sampling or, what is more likely, the fish reduced its breathing during the sampling procedure.

In oxygen samples taken by Black et al. (1966) on unexercised brook trout (*Salvelinus fontinalis*) during winter at a temperature of 0 C, the oxygen contents were 4.3 and 4.7 vol per cent in two samples representing oxyhemoglobin contents of 3.2 and 3.6 vol per cent, respectively. The values reported in this paper are lower. However, in four samples taken during summer by Ferguson and Black (1941) in unexercised rainbow trout scarcely any oxygen was left in the blood samples taken at a temperature of 15–22 C. The oxygen content dropped sharply during severe exercise then rose to the highest values in the first hour of recovery at which time the average was 4.28 vol per cent. During the next 23 hr of recovery, the values fluctuated but were never less than the initial unexercised value. The values during recovery show two low dips namely at the 8th and 12th hours. As will be shown later there are also marked variations in lactate during the recovery period reported in previous studies by Black (1957a).

Of some interest is the value for oxygen reported for the steady state, i.e. fish swimming in the rotating annulus. The average value of 1.1 vol per cent is not very different from the average of the unexercised value.

In all instances the true values of the oxygen content of the blood are probably higher, for the very act of sampling may have reduced the oxygen level by possible interference with normal gill ventilation.

Pyruvate

Pyruvate present in blood taken from the heart under various conditions is presented in Table I and Fig. 2. The average unexercised value was 0.45 mg/100 ml (i.e. milligrams per 100 ml blood) a value which is one and a half times as great as the average value of 0.29 $\pm$ 0.029 mg/100 ml reported by Black et al. (1962) for the same condition.

The time course of pyruvate (Table I, Fig. 2) during the period of severe exercise and the 24 hr of recovery thereafter, is similar to that observed by Black et al. (1962). Pyruvate determined for moderate exercise for 20 min in the rotating annulus averaged 0.642 mg/100 ml a value which is slightly higher than the average of 0.55 $\pm$ 0.077 mg/100 ml reported earlier by Black et al. (1962).

Lactate

Changes in the blood level of lactate during strenuous exercise and recovery shown in Table I, and Fig. 2 are very similar to the results obtained on rainbow trout by Black et al. (1962).

The average value for the condition of moderate exercise was 26.05 mg/100 ml, a value which is twice as great as that observed by Black et al. (1962) for trout which had been subjected to moderate exercise for a period

TABLE I. Body weights, sampling times, and venous blood levels of hemoglobin, oxygen, pyruvate, l dioxide exercise, eight periods of recovery following 15 min strenuous exercise, state, i.

		Unexercised	Severe exercise				
			2 min	5 min	9 min	15 min	30
Body Weight (g)	Mean±SE (*n*) Range	186.8±9.625 (12) 139–259	190.5±19.84 (8) 131–248	201.8±7.69 (8) 172–231	188.9±6.66 (8) 115–297	183.1±9.68 (8) 136–212	214.7 (147
Sampling time (*sec*)	Mean±SE (*n*) Range	26.2±4.624 (12) 10–63	16.0±6.796 (8) 5–63	14.4±3.38 (8) 5–30	30.6±12.953 (8) 4–110	19.6±3.505 (8) 7–34	22.1 (5
Hemoglobin (*g per cent*)	Mean±SE (*n*) Range	8.007±.188 (12) 7.03–8.99	9.130±.418 (8) 7.88–10.9	9.095±.344 (8) 7.82–10.5	9.590±.288 (8) 8.36–10.8	10.08±.380 (8) 8.56–11.6	9.08 (8.34
Oxygen (*vol per cent*)	Mean±SE (*n*) Range	1.56±.350 (12) .13–3.6	.570±.251 (8) 0–1.9	.291±.108 (8) 0–.74	.414±.155 (8) 0–1.0	.115±.076 (8) 0–.51	2.4 (.98
Pyruvate (*mg/100 ml*)	Mean±SE (*n*) Range	.452±.025 (11) .36–.61	.544±.050 (7) .41–.78	.601±.066 (7) .35–.92	.926±.104 (7) .66–1.3	.693±.042 (8) .51–.90	1.61 (1.2
Lactate (*mg/100 ml*)	Mean±SE (*n*) Range	5.099±.452 (9) 2.48–7.21	24.76±1.138 (8) 20.1–28.7	42.56±3.853 (7) 32.3–62.6	59.85±5.591 (8) 28.5–81.5	69.71±5.757 (8) 44.0–93.0	110.5 (76.0
Carbon dioxide (*vol per cent*)	Mean±SE (*n*) Range	29.47±1.039 (12) 24.0–33.8	29.91±2.125 (8) 20.6–38.8	25.68±1.624 (8) 17.0–31.7	31.44±2.648 (8) 18.7–40.9	26.35±1.471 (8) 20.9–31.2	21.96 (14.8
Excess lactate (mM/litre)			2.07	3.97	5.48	6.87	10.3

dioxide, and excess lactate in 1½-year-old rainbow trout for controls, four periods of strenuous state, i.e. trout swimming in a rotating annulus at a steady sustained speed.

	Recovery period						Steady state
ar	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	
±5.69	211.2±13.81	199.1±15.12	213.5±20.51	236.4±14.64	234.5±9.74	189.9±9.34	138.0±8.55
9)	(9)	(8)	(8)	(10)	(11)	(8)	(9)
267	132–270	158–292	165–338	160–319	179–305	154–231	102–176
±1.645	17.7±4.555	29.8±8.066	16.4±2.398	29.0±5.989	21.2±3.322	19.3±3.584	16.7±2.693
9)	(9)	(8)	(8)	(10)	(11)	(8)	(9)
43	4–43	13–81	8–25	8–62	8–48	9–35	4–25
±.368	9.277±.285	9.515±.239	9.446±.393	9.416±.518	8.501±.317	9.149±.351	9.156±.322
9)	(9)	(8)	(8)	(10)	(11)	(8)	(8)
11.8	8.05–10.6	8.62–10.7	7.94–10.8	7.09–12.6	6.97–10.6	7.91–10.4	7.48–10.3
±.503	2.09±.528	3.11±.490	2.84±.283	2.03±.230	3.46±.314	3.85±.328	1.10±.367
9)	(8)	(8)	(7)	(8)	(11)	(8)	(8)
6.5	0–4.5	1.2–5.5	1.9–4.0	.97–2.8	2.4–5.5	2.2–5.1	0–2.8
±.094	1.71±.100	1.27±.092	1.10±.099	.892±.105	.578±.043	.560±.019	.642±.026
0)	(8)	(8)	(8)	(9)	(10)	(8)	(9)
2.1	1.3–2.1	.95–1.7	.76–1.5	.40–1.4	.37–.75	.49–.63	.51–.78
±6.64	155.1±4.48	98.75±12.324	44.23±7.288	19.47±1.307	11.73±1.597	3.783±.845	26.05±1.57
9)	(7)	(8)	(8)	(7)	(11)	(7)	(8)
152	143–169	43.9–148	21.6–82.0	16.5–24.8	3.31–19.9	.682–7.30	20.3–32.7
±1.612	18.51±1.458	22.71±1.105	25.91±1.368	31.05±1.321	28.11±1.267	26.23±1.585	26.65±1.810
9)	(9)	(8)	(8)	(10)	(11)	(8)	(8)
24.2	11.6–27.1	18.3–27.7	19.7–30.9	24.1–38.5	22.3–35.0	20.6–33.6	20.9–36.5
	15.1	9.37	3.53	1.05	0.578	−0.281	2.09

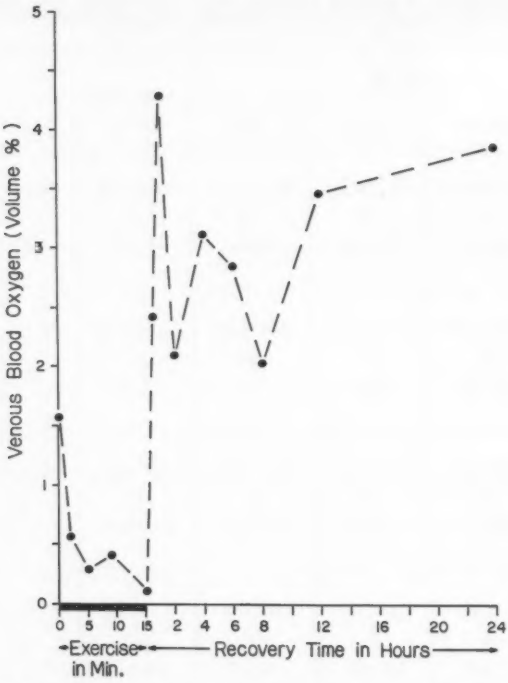

FIG. 1. Venous blood oxygen levels in 1½-year-old rainbow trout for various periods of severe exercise and recovery up to 24 hr following 15 min strenuous exercise.

of 30 min. There was no marked difference in the weights of these two groups of trout.

CARBON DIOXIDE

The carbon dioxide content of venous blood declined from the initial resting value of 29.47 vol per cent to 26.35 vol per cent at the end of 15 min strenuous exercise. During the recovery period carbon dioxide declined for the first 2 hr to a value of 18.51 vol per cent and then increased to a peak value of 31.05 at the eighth hour (Table I, Fig. 2). These results are remarkably similar to the changes reported by Black et al. (1959) for equivalent conditions. The average carbon dioxide level after 20 min of moderate activity was 26.65 vol per cent a value which is not significantly different from the initial unexercised value ($p > 0.05$). Auvergnat and Secondat (1942) reported that the blood level of carbon dioxide in carp declined from a resting value of 35 vol per cent to 17.5 vol per cent at the end of a 15-min period of severe exercise and remained as low as 16 vol per cent till the end of the 6th hour of recovery.

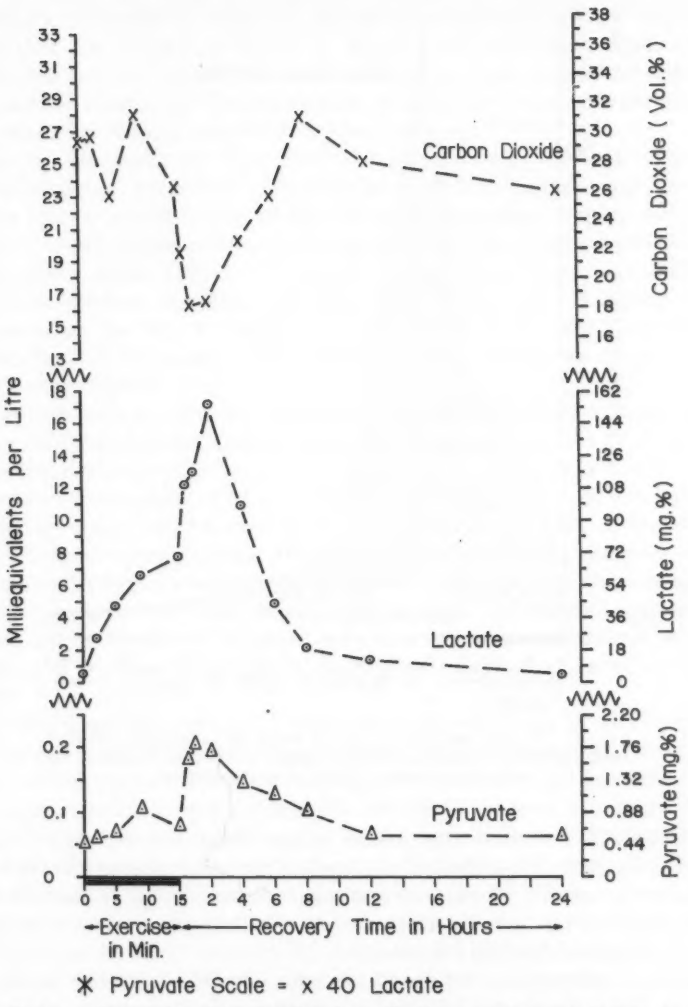

FIG. 2. Venous blood levels of carbon dioxide, pyruvate and lactate in 1½-year-old rainbow trout for various periods of severe exercise and recovery up to 24 hr following 15 min strenuous exercise.

DISCUSSION

During severe exercise the depletion of muscle glycogen in rainbow trout is extremely rapid, one-half of the resting level being utilized in the first 2 min of vigorous activity (Black et al., 1962). In experiments carried out on different fish but in the same study, they demonstrated further that the transfer of both lactate and pyruvate from muscle to blood begins almost immediately.

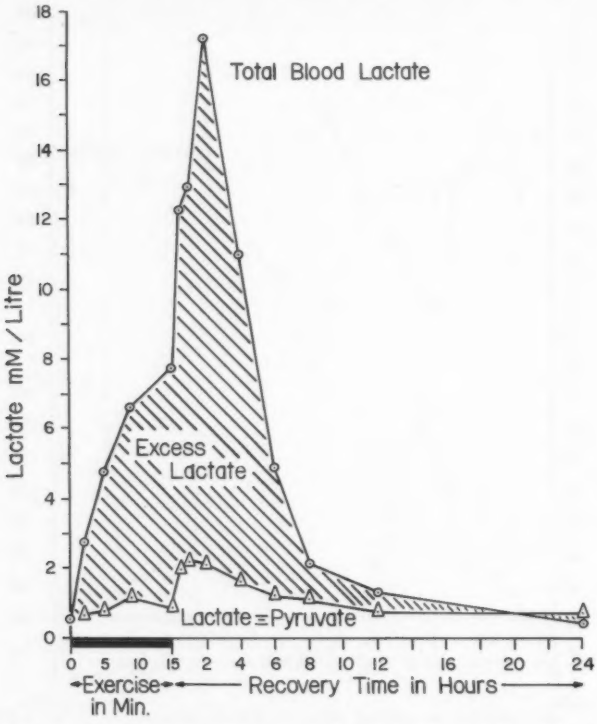

FIG. 3. "Excess lactate" in venous blood of 1½-year-old rainbow trout subjected to various periods of severe exercise and recovery up to 24 hr following 15 min of severe exercise.

Black (1957) showed that blood lactate increased immediately in fish vigorously exercised regardless of the level of the oxygen supply in the external aquatic environment. The interpretation of these findings is that circulation did not support the aerobic energy cycle with the necessary promptness to supply the sudden demand for energy.

In the present study, all data have been obtained from blood taken from the heart. This means that all interpretations must be referred to the condition of the venous blood as it converged and returned to the heart after flowing through all the tissues of the body except the gills. From previous work of Black et al. (1962) the disparity between the levels of pyruvate and lactate in the muscle and blood is very great, e.g. the level of pyruvate in the muscle at the end of 15 min severe exercise was an average of 3.94 ± 0.658 mg/100 g tissue, whereas the blood level was 0.51 ± 0.04 mg/100 ml blood; the muscle lactate at the end of 15 min strenuous activity average 295 ± 27.1 mg/100 g tissue compared to an average of 54.0 ± 5.98 mg/100 ml in blood. In all these cases, the sample of blood and muscle was taken from the same fish. There are no

observations on levels of oxygen or carbon dioxide for tissues of fish, but it is likely that the oxygen level will be much lower than the oxygen content of blood, and that the carbon dioxide level may be more or less dependent on the immediate history of the production of hydrogen ions and the capacity of the tissues for the neutralization of the acidic ion.

Use can be made of Huckabee's "excess lactate" concept to provide more information regarding relationships between lactate and pyruvate and from this, some indication of the status of the oxygen supply supporting the Krebs cycle. These relationships for rainbow trout have been discussed fully by Black et al. (1962) who noted in their data that "excess lactate" disappeared between the 8th and 12th hour of recovery following 15 min severe exercise. In Fig. 3 "excess lactate" values have been portrayed for data obtained in this study. The results are very similar to those reported by Black et al. (1962).

Pyruvate and lactate are important metabolites which appear in both anaerobic and aerobic metabolism; however, if the combustion of metabolites through the Krebs cycle is impaired through a reduction in the oxygen supply then lactate accumulates. The accumulation of lactate, then, is one important indication of a disturbed homeostasis, i.e. fatigue. In a study of the blue gill sunfish (*Lepomis machrochirus*) Heath and Pritchard (1962) studied changes in lactate and oxygen consumption in the recovery period following vigorous activity. A true oxygen debt was caused by this activity and was not paid for some hours. The level of blood lactate returned to the unexercised level in 10 hr and the level of oxygen consumption returned to unexercised levels in 24 hr.

It would appear then that changes in blood levels of the respiratory gases, oxygen and carbon dioxide, are linked with changes in the three carbon units of carbohydrate metabolism during severe exercise and recovery. During the 15-min period of severe exercise the venous blood level of oxygen dropped sharply (Table I, Fig. 1). During this same period pyruvate and lactate levels rose while carbon dioxide levels fluctuated widely and unevenly (Table I, Fig. 2). Indeed, at the end of 9 min of severe exercise the average carbon dioxide value was actually higher than the initial resting value. Similar erratic changes in carbon dioxide were observed by Black et al. (1959). This rapid fall in the oxygen level of venous blood is due to the intense utilization of oxygen by the respiring tissues which is promptly followed by an increased extraction of oxygen from the blood returning from the gills. The stores of muscle glycogen are quickly depleted (Black et al., 1962) and this is accompanied by an increase in pyruvate and lactate levels in the blood due to the rapid increase in the concentration of these substances within the muscles. At the same time the "excess lactate" has greatly increased. Black et al. (1959) related variations in carbon dioxide during a 15-min period of severe exercise in 1½-year-old Kamloops trout (*Salmo gairdneri*) to changes which may occur in creatine phosphate as demonstrated by Felton (1956) in 3-year-old hatchery-bred steelhead trout.

During recovery the oxygen level of venous blood ascended from 0.115 vol per cent at the end of 15 min severe exercise to 2.243 vol per cent at the end of the first ½ hr of recovery, a value which is slightly greater than the initial unexercised level. While there was a wide fluctuation during the remaining 24 hr of recovery, the average oxygen level was always above the initial unexercised level. Whatever the degree of circulation it would appear that the oxygen tension in the blood did not drop below 16 vol per cent during the 24 hr of recovery. Using the data of Black et al. (1966) for brook trout (*Salvelinus fontinalis*) at 15 C this would mean that the oxygen tension did not dip below 8 mm Hg pO_2. Subjectively, the trout did not appear to be unduly active while being held in the recovery basket; the assumption is made that no undue exercise occurred during the recovery period. Thus, the oxygen extracted from the blood during the circuit between the gills and the tissues could be used to take care of resting metabolism and to repay the oxygen debt manifested by the presence of "excess lactate." A precise evaluation of these facts cannot be given, for both the extent of changes in muscle lactate and possible spurts of activity are unknown.

The level of lactate was always in excess of the pyruvate during recovery. However, both declined to approximate the unexercised levels by the 12th hour of recovery (Table I, Fig. 2). Even by the 8th hour "excess lactate" had declined to nearly the unexercised level. Presumably, adequate oxygen would reverse the direction of the equilibria so that lactate would be reconverted to pyruvate, and pyruvate would be metabolised through the Krebs cycle yielding energy, carbon dioxide, and water. Black et al. (1966) noted that during the period of recovery, blood glucose levels did not increase markedly. They also observed that both muscle and liver glycogen declined (Black et al., 1960, 1962). In other words it appeared that carbohydrate metabolism did not follow the cori cycle formerly claimed to be characteristic in mammals (Drury and Wick, 1956). Lactate appeared to be metabolised in the muscles in trout rather than being transported to the liver and synthesised into liver glycogen as in the mammal. Of very great interest is the recent observations by Wittenberger and Diaciuc (1965) that glycogen decreases, etc. in the white muscles and that lactate diffuses from the white muscles to be burned in the adjacent red muscles.

During recovery, the carbon dioxide level fell from 26.36 vol per cent at the end of 15 min severe exercise to 18.36 vol per cent at the end of 1 hr of recovery (Table I, Fig. 2). Following the 2nd hour, the carbon dioxide ascended steadily to a level of 31.05 vol at the 8th hour of recovery (Table I, Fig. 2). Black et al. (1959) reported a similar but less striking series of changes for carbon dioxide in Kamloops trout. As reported previously (Black et al., 1959), blood lactate continued to rise from a value of 69.71 mg/100 ml at the end of 15 min severe exercise to 155.1 mg/100 ml at the end of the 2nd hour of recovery, whereupon the lactate dropped to 19.47 mg/100 ml at the end of the 8th hour of recovery. The pyruvate changes were similar to lactate but quantitatively much less.

It would appear that carbon dioxide was displaced by high levels particularly of lactate diffusing into the bloodstream from the muscles and that as lactate declined carbonate increased to maintain the total population of anions in the blood, a level to match the cation population.

Black (1957b), Parker and Black (1959), and Parker et al. (1959) observed that death from severe activity occurred in various species of Pacific salmon between the 1st half hour and the 2nd hour of recovery, and that in all cases blood lactate was in relatively high concentrations during this interval of recovery. Presumably, the hydrogen ion concentration of the blood may be a factor in the death of the fish.

The concentrations of oxygen and carbon dioxide in the blood of fish which had been swimming at a steady state in the rotating annulus, while lower did not differ significantly from the unexercised values. However, blood pyruvate was about half again as great and the lactate value five times greater than the respective resting values. "Excess lactate" was 2.09 mM/litre, a level almost identical to the initial unexercised level.

SUMMARY

1. Hemoglobin levels were determined for all conditions investigated. There was a significant increase in hemoglobin to an average value of 10.1 g/100 ml blood following 15 min of severe exercise ($p < 0.05$).
2. The venous blood oxygen level declined from a resting value of 1.56 to 0.115 vol per cent following 15 min severe exercise. All average values recorded during recovery were equal to or greater than the initial unexercised value.
3. The venous blood level of pyruvate increased during strenuous exercise from a resting value of 0.452 to 0.693 mg/100 ml following 15 min of strenuous activity. During recovery there was a further and significant increase to a peak level of 1.79 mg/100 ml at the end of the 1st hour. Pyruvate levels thereafter steadily declined after the 2nd hour to approximate the initial unexercised level at the 12th hour of recovery.
4. The blood lactate level of venous blood increased from initial unexercised value of 5.099 mg/100 ml to a value of 69.71 mg/100 ml following 15 min of severe exercise. During recovery the blood level continued to rise to a level of 155.1 mg/100 ml and then sharply declined during the first 6 hr to approximate the initial resting level at the end of the 24 hr.
5. Alterations in carbon dioxide levels of venous blood were erratic. The initial resting level averaged 29.47 vol per cent and the level at the end of 15 min strenuous exercise was 26.35 vol per cent. During the recovery period, however, there was a steep decline for the first 2 hr to a value of 18.51 vol per cent followed by a progressive rise to approach the initial unexercised level by the 8th hour.
6. The results of fish swimming steadily in the rotating annulus revealed no significant change for the unexercised state for both oxygen and carbon

dioxide levels in venous blood. However, both the pyruvate and the lactate increased during the steady state.

7. The results reported above corroborate earlier studies. In the present paper all the constituents were determined on the same sample of venous blood. Clearly there is a close relationship between the blood levels of pyruvate and lactate, the latter, however, being 10-fold the concentration for any one condition. There is also an inverse correlation between these acids of carbohydrate metabolism and the venous blood level of oxygen. Venous blood levels of carbon dioxide while erratic during strenuous exercise declined while pyruvate and lactate ascended in the first 1–2 hr of recovery. The carbon dioxide level rose to near normal levels at the end of the 8th hour at which time the pyruvate and lactate had fallen. The inverse correlation is seen very clearly when "excess lactate" levels are examined. The significant increase in the level of hemoglobin after 15 min severe exercise reflect that osmotic shifts brought about by the creation of new particles by the breakdown of muscle glycogen. The results obtained depict close interrelationships between gaseous and carbohydrate metabolism of the blood during and following severe muscular exercise.

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Biological Characteristics of a Herring Population on the South Coast of Newfoundland[1]

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ABSTRACT

A study of herring was undertaken at the onset of a period of increased exploitation. Catches were examined for length and age composition, growth rates, length–weight relationship, sex, maturity, and fatness.

Mean length (33.0 cm) and mean age (8.2 years) were smaller than recorded from earlier investigations, but growth rates have not changed appreciably. Winter-caught herring are of poor quality ranging in fat content between 2.3 and 7.7% of their wet weights.

An increase in the proportion of herring with immature gonads as the season progresses and a complete gradation of otolith types from spring-spawned to fall-spawned types suggests: (1) exploitation of fish that previously did not enter the fishery until later in the season, and (2) a spawning season that extends over the spring, summer, and autumn months.

INTRODUCTION

In November 1964, the MV *Lavallee*, a Pacific coast purse-seiner, was introduced to the herring fishery on the south coast of Newfoundland to supply herring for oil and meal to the new British Columbia Packers Ltd. reduction plant at Harbour Breton, Nfld.

Between February 3 and May 31, 1965, the *Lavallee* caught an estimated 3,758,000 lb of herring in Area J (Fig. 1). During the same period in 1964, the total catch from Area J was 930,916 lb, taken mostly by gill nets.

The purpose of the present investigation was to record biological characteristics of the herring stocks at the onset of a period of increased rate of exploitation. Data are presented on the length and age composition of the stocks, growth rate, length–weight relationship, sex and maturity, and fatness or condition. Wherever possible, these data are compared with similar data obtained during previous studies by other investigators. However, the data are primarily intended to serve as a basis for future comparisons to indicate the effect of an increased rate of exploitation on a stock of herring.

MATERIALS AND METHODS

The MV *Lavallee* searched for herring with a Simrad Model 512-11 echo-sounder from Fortune Bay to Port-aux-Basques, but encountered and caught fish only in Baie de Vieux and the immediate vicinity (Fig. 1). Samples of

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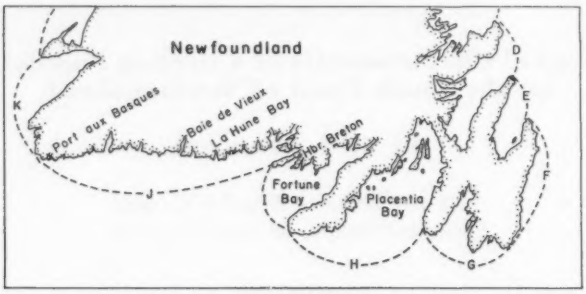

FIG. 1. Locations of places and Newfoundland sea fisheries areas referred to in the text.

the catches were obtained throughout the period January 24 to March 6, 1965. Each sample consisted of either 50 or 100 fish and a total of 1200 herring was examined, all in the fresh condition.

Herring were measured to the nearest millimetre from the tip of the lower jaw with the mouth closed to the end of the longer lobe of the caudal fin extended straight back in line with the body. Length composition data were plotted in half-centimetre groupings with centimetre and half-centimetre lengths as the group mid-points.

Scales and otoliths were taken from all fish sampled. Age determinations were made from the otoliths of all 1200 of the herring. Scales from 400 herring were read as a check on the otolith age determinations.

Gonads were examined for determination of sex and sexual maturity. The state of gonad development was divided into four stages as follows: *Stage A* — Immature — gonads small, flattened and pinkish in appearance, to one-quarter full; *Stage B* — Maturing — one-quarter full to full; *Stage C* — Ripe and running; *Stage D* — Spent (ovaries with residual eggs; testes loose and bloodshot).

Fatness was determined on either one fish or on a sample of six fish (three males and three females), ground to a smooth paste in a meat grinder.

Prepared samples were mounted in a Soxhlet extraction apparatus When the extraction was complete, the per cent fat content of the wet weight of the whole fish was obtained.

LENGTH AND AGE COMPOSITION

Herring ranged in total length from 19.1 to 41.0 cm and in age from 4 to 16 years. Ages determined by scale reading were in agreement with those by otolith reading. Mean length of the 1200 fish sampled during the investigation was 33.0 cm and mean age was 8.2 years. Only 18% were 10 years of age or older (Fig. 2).

Tibbo (1957b) recorded mean lengths of herring from the south and west coasts of Newfoundland varying from 34.4 to 36.1 cm and mean ages from

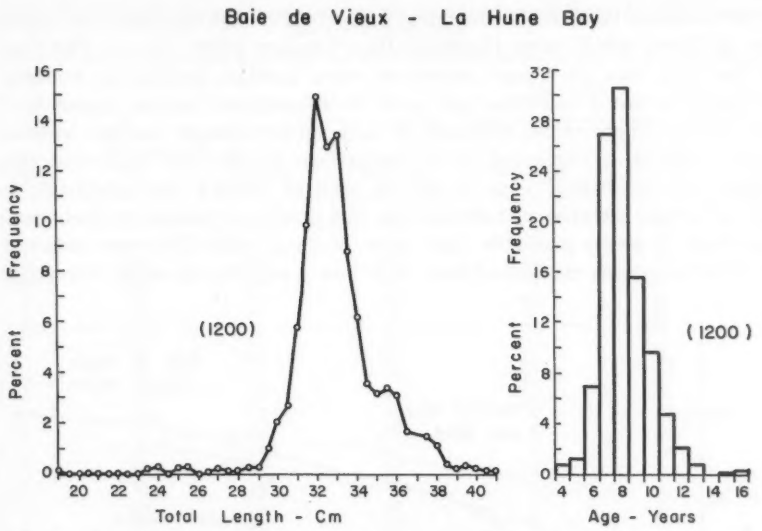

FIG. 2. Length and age composition of herring samples from the Baie de Vieux–La Hune Bay area, January 24–March 6, 1965.

10.9 to 13.0 years. He also concluded on the basis of differences in morphological and meristic characteristics that there are several separate and distinct herring populations in the areas he studied.

In contrast to Tibbo's conclusion, Olsen (1961) described a trend of geographical segregation with age on the south coast, with a westward distribution of the large and old fish, and a concentration of the younger age-groups in the east in Placentia Bay.

Both Tibbo's and Olsen's data suggest that one would expect larger and older herring from Baie de Vieux than were encountered in the present investigation. However, the samples from Baie de Vieux cannot be considered representative of any more than the herring stocks in that immediate vicinity.

RATE OF GROWTH

Day (1957a,b) and Tibbo (1957a,b) analyzed length and age data from various areas in the Gulf of St. Lawrence and the Atlantic coast of Nova Scotia for differences in rates of growth of herring. There was some evidence that growth in the Newfoundland area is similar to that in the southern Gulf, but more rapid than in the estuary of the St. Lawrence.

Olsen (1961) found the growth rate of herring in Newfoundland waters to be fairly uniform from one area to another and considerably higher than that found by Tibbo (1956).

Mean length-at-age data from the 1965 Baie de Vieux–La Hune Bay samples were compared with those of Jean (1953) obtained at Grande-Rivière

in the southern Gulf of St. Lawrence from the 1952 spring-spawning stock and that of Olsen (1961) from Placentia Bay, January 1958 (Fig. 3). The Grande-Rivière and Baie de Vieux curves are very similar, tending to confirm the similarity between southern gulf and Newfoundland herring noted by Day and Tibbo. There is no evidence of any major change having occurred in growth rate as is suggested by a comparison of the 1965 data and Olsen's. Adding one additional year of age to each of Olsen's age categories would tend to reduce the observed differences, but would not eliminate them entirely. Therefore, it seems probable that there is some basic difference between the age determinations made by Olsen and those made by the other investigators.

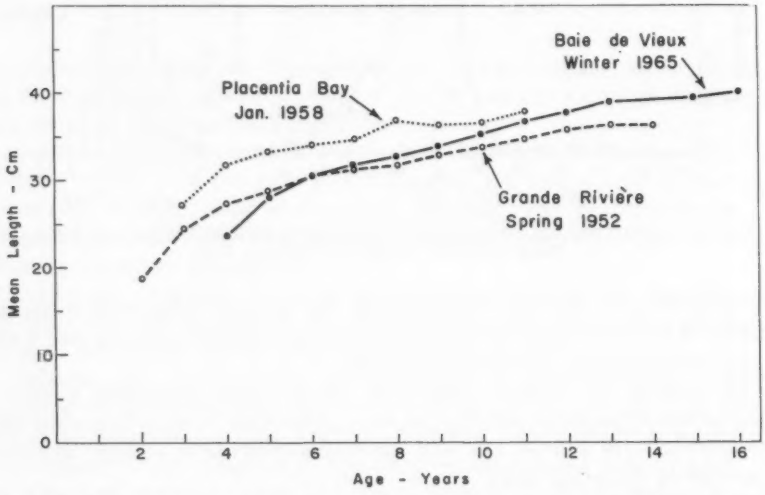

FIG. 3. Rates of growth of herring from (A) Placentia Bay, January 1958, (B) Baie de Vieux, winter 1965, and (C) Grande-Rivière, spring 1952.

SEX AND MATURITY

Table I summarizes the sex and maturity data collected in the Baie de Vieux–La Hune Bay area.

Only three out of 17 samples contained more males than females and a 1:1 sex ratio appears in only one sample. The overall sex proportion for these samples (42.8% males) indicates a considerable excess of females.

Of the 1200 fish sampled between January 24 and March 6, 84.0% had immature gonads, 15.8% were maturing, 0.2% were ripe and running, and none was recently spent.

As the season progressed, there was an obvious increase in the proportion of immature individuals and a decrease in maturing fish. This would seem to indicate that as the fish mature, they leave the areas being fished by purse-seine.

TABLE I. Sex proportions and maturity of herring from the Baie de Vieux–La Hune Bay area, January–March 1965.

Date	No. of fish	Males	Maturity			
			Immature	Maturing	Ripe and running	Spent
		%	%	%	%	%
Jan. 24	100	47	63	36	1	0
Jan. 31	100	46	80	20	0	0
Feb. 5	50	38	60	40	0	0
Feb. 6	100	52	72	28	0	0
Feb. 7	100	46	57	41	2	0
Feb. 13	50	38	86	14	0	0
Feb. 14	50	28	78	22	0	0
Feb. 15	50	54	90	10	0	0
Feb. 19	50	42	98	2	0	0
Feb. 21	50	52	92	8	0	0
Feb. 22	50	46	92	8	0	0
Feb. 19–22	50	50	90	10	0	0
Feb. 25	50	46	96	4	0	0
Mar. 2	100	35	95	5	0	0
Mar. 2	50	40	94	6	0	0
Mar. 6	100	31	93	7	0	0
Mar. 2–6	100	38	93	7	0	0
Average:		42.8	84.0	15.8	0.2	0.0

Prior to 1949, all reports of observations indicated that Newfoundland herring were spring spawners. However, Olsen (1961) concluded that "some time during the last 10–12 years, the Newfoundland herring, which for at least 60 years had been spawning in the spring or early summer, changed their spawning habits so that now some spawning seems to occur in practically every month of the year." Certainly, fish with immature gonads in March (as observed in the present investigation) would not be in spawning condition by May or June and probably would not spawn until fall. But, the decreasing numbers of maturing fishes observed in the catches between January and March 1965 most likely represent a portion of the stocks which spawn in spring or early summer. An examination of the otoliths following the method of Parrish and Sharman (1958) indicated a range of nucleus types from the clearly defined spring-spawned type to the fall-spawned type with a complete gradation of nucleus types in between. Thus, there is indirect evidence at least to strengthen Olsen's contention that there is "an extreme spread in spawning time" in Newfoundland waters.

FATNESS AND LENGTH–WEIGHT RELATIONSHIP

Herring samples from Baie de Vieux taken during the month of February were analyzed for fatness or condition. Fatness was expressed as percentage fat content of the wet weight of the whole fish. Whole fish weighed between

242 and 275 g. Percentage fat content ranged from 2.3 to 7.7%. In comparison, the mean fat content of herring caught in Nova Scotia waters during June ranged from 9.7 to 14.8% and in July from 12.5 to 17.3% (unpublished data).

In addition to length measurements, wet weights in grams were recorded for 750 individuals. These data were used to obtain a relationship of length to weight for winter-caught herring, using an equation of the type

$$W = al^k$$

where W = wet weight of whole fish in grams and l = total length in centimetres
to give the values:

$$W = 0.017994\ l^{2.71}$$

The theoretical curve is shown in Fig. 4.

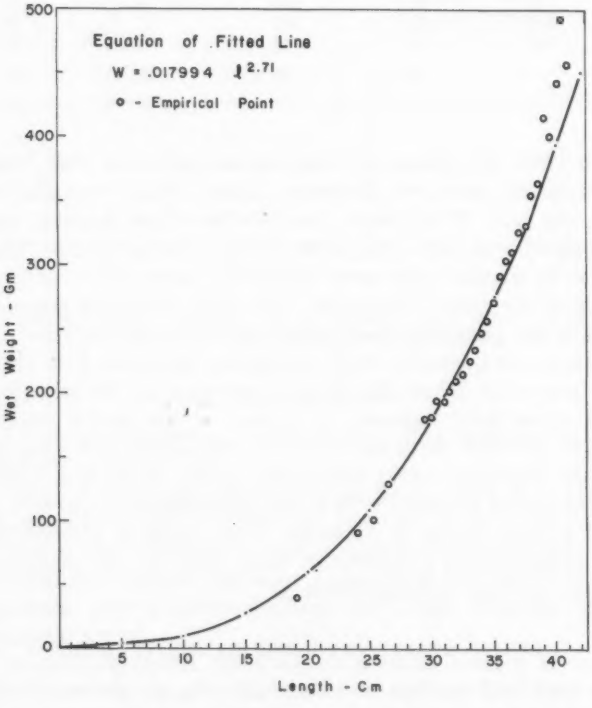

FIG. 4. The length–weight relationship for winter-caught herring from the Baie de Vieux–La Hune Bay area.

DISCUSSION

In past years, the herring fishery on the south coast of Newfoundland was based on aggregations of prespawning fish that appeared in inshore areas during the late fall and early winter. Fishing was carried on close to shore with small purse-seines, shore seines, traps, and gill nets.

With the introduction of a large Pacific coast purse-seiner, it has become necessary to fish further offshore in deeper water to avoid fouling the gear on the bottom. The fishery is still executed within the sheltered waters of the bays, but in depths of from 30 to 150 fath, setting the gear on schools swimming at depths of between 7 and 18 fath.

Previously, commercial catches on the south and west coasts consisted chiefly of large and old herring with mean lengths of 34.4–36.1 cm and mean ages from 10.9 to 13.0 years (Tibbo, 1956). In contrast, a mean length of 33.0 cm and a mean age of 8.2 years was recorded for samples taken from Baie de Vieux–La Hune catches during the winter of 1965. This change in size and age composition may indicate the exploitation of an entirely different population of herring; it may be the result of exploiting a younger portion of the same population; or, the stock itself may have a younger age composition due to year-class strength variations.

Tibbo (1957b) recorded herring caught during January–February 1947 in Fortune Bay as 6.2% immature individuals, 93.1% maturing, 0.2% ripe and running, and 0.5% spent. In March–April 1948, immature fish made up 8.9% of the samples and 91.1% were maturing. By May–June 1948, only 0.4% were immature, the proportion of maturing fish had dropped to 55.7%, 27.0% were ripe and running, and 16.9% were recently spent. It was concluded that spawning occurred in late spring or early summer. More recently, however, Olsen (1961) found evidence of herring spawning in practically every month of the year and concluded that there had been a change in the spawning habit of Newfoundland herring.

The overwhelming proportion (84.0%) of immature herring occurring as late as March in the catches of 1965 is further evidence that spawning is not limited to late spring or early summer.

Perhaps of greater importance is the fact that these are the first records of such a large proportion of immature individuals in winter catches on the south coast. Also, a noticeable increase in the proportion of immature fish and a decrease in maturing individuals as the season progressed indicated that as the fish matured they left the area being exploited by the purse-seine gear.

No data from a concurrent inshore fishery were obtained during the 1965 study period. However, it was shown that as the fish matured they left the area being exploited by the purse-seine gear. Furthermore, in previous years when herring were taken by gill net and beach seine close to shore, the catches consisted of fish in a more advanced stage of sexual maturity. It is probable, therefore, that the use of the large Pacific coast seining gear

has resulted not in the exploitation of new stocks, but in the earlier exploitation of fish that previously did not enter the fishery until later in the season.

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A New Species of *Bradyidius* (Copepoda: Calanoida) from the Pacific Coast of North America[1]

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ABSTRACT

A new species *Bradyidius saanichi* from Saanich Inlet, Vancouver Island, British Columbia, Canada, is described and illustrated in detail. This species is closely related to *B. pacificus* (Brodsky, 1950) among the six previously known species in the genus, but can be readily distinguished from the latter by the strongly divergent rostral rami in addition to some other differences.

INTRODUCTION

The new species of *Bradyidius* described below was found in plankton samples collected in Saanich Inlet, Vancouver Island, B.C., by the collecting vessel *Hydah* of the Friday Harbor Laboratories in September 1965. This is the seventh species of the genus, and the six previously known species are as follows: *B. armatus* Giesbrecht, 1897; *B. similis* (G. O. Sars, 1902); *B. tropicus* (Wolfenden, 1905); *B. pacificus* (Brodsky, 1950); *B. arnoldi* Fleminger, 1957; *B. angustus* (Tanaka, 1957). See Wilson (1950) for the treatment of the generic name.

Bradyidius saanichi, n. sp. (Fig. 1–3)

Types. Holotype female, U.S. National Museum No. 113241; allotype male, U.S.N.M. No. 113242; 5 paratypes of each sex, U.S.N.M. No. 113243. Type locality, Saanich Inlet, Vancouver Island, British Columbia, Canada, 48°40′N lat, 123°30′W long.

Female. The total length from the anterior end of the cephalosome to the end of the caudal ramus ranges from 2.33 to 2.56 mm according to 37 measurements. The metasome is about 3.4 times the length of the urosome. The 2nd thoracic segment (of the 1st swimming legs) is completely fused with the cephalosome. The 5th and 6th thoracic segments are also completely fused. The body is strongly built with the forehead in lateral aspect smoothly curved into a rather small rostrum. There is a distinct construction at the base of the rostrum, from which arise 2 small sensory hairs. The rostrum (Fig. 1D) is bifurcated into strongly divergent rami (about 45° in respect to the mid-sagittal plane of the body). The postero-lateral margin of the metasome is produced at either side into a strong, acute point directed postero-dorsad and reaching nearly to the distal end of the genital segment.

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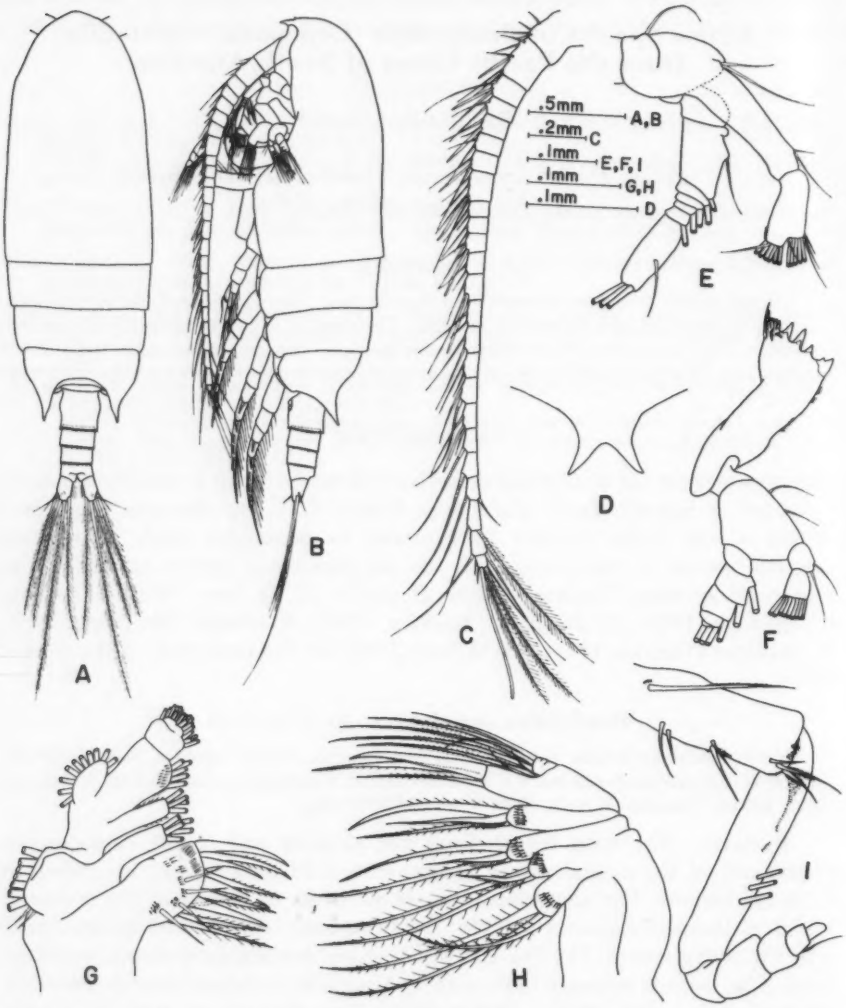

FIG. 1. *Bradyidius saanichi*, n. sp. Female: A, habitus, dorsal view; B, habitus, lateral view; C, antennule; D, rostrum; E, antenna; F, mandible; G, maxillule; H, maxilla; I, maxilliped.

The urosome is 4-segmented; the segments and the caudal rami have the following proportional lengths, from anterior to posterior: 37:15:14:15:19. The caudal rami are longer than wide (5:3), with 4 long terminal setae, a short external seta, and a short ventral seta. The 2nd terminal seta from inside is about 1.5 times longer than the other three which are subequal in length.

The antennules (Fig. 1C) reach the end of the metasome when folded back, and are 24-segmented. Both rami of the antenna (Fig. 1E) are about equal in length. The exopod is 7-segmented; the 1st segment is without setae, while the 2nd segment bears a small protuberance at the proximal end of the internal margin and 2 setae distally on the same margin. Each of the 3rd to 6th segments carries a single seta and the 7th segment 4 setae.

The mandible (Fig. 1F) is provided with a strong masticatory blade which is armed with 8 separate teeth plus a basal seta. The basis is grossly triangular, bearing 2 setae along the internal margin. The maxillule (Fig. 1G) is well developed with a powerful gnathobase carrying 14 strong spines. The setal arrangement on the various lobes of the appendage is best described by reference to the figure.

The maxilla (Fig. 1H) has 5 prominent lobes in addition to a small endopod. Each of the 1st to 4th lobes carries 3 strong, spinulose setae and has 2 transverse rows of small spinules on the posterior surface. The 5th lobe bears a strong, dagger-shaped spine in addition to 2 spinulose setae.

The maxilliped (Fig. 1I) consists of a slender basipod and a short endopod. The coxa carries 4 groups of setae, composed of 1, 2, 2, and 3 setae, respectively. The basis bears 3 setae just distad of the midpoint and 2 setae at the distal end. The endopod is 5-segmented, carrying 4 setae on each of the 1st and 2nd segments, 3 setae on the 3rd segment, and 4 setae on each of the 4th and 5th segments.

The 1st pair of legs (Fig. 2A) has 3-segmented exopods and 1-segmented endopods. The endopod reaches the distal end of the 2nd exopodal segment, and bears a round tubercule on the external margin. The distal half of the tubercle is densely covered by small spinules. There is also found a patch of spinules on the anterior surface of the endopod close to the insertion of the 2nd internal seta. Each segment of the exopod bears an external spine. The 1st spine is much thinner than the other 2 and reaches closely to the proximal end of the 2nd. The 2nd spine is slightly longer than the 1st and extends a little beyond the proximal end of the 3rd. The 3rd spine is about twice as long as the 2nd and more than one-half the length of the terminal seta of the same segment.

The 2nd leg (Fig. 2B) has a 2-segmented endopod and a 3-segmented exopod. The 3rd and 4th pairs of legs (Fig. 2C, D) are similar. Both the exopods and the endopods are 3-segmented. There are found a few spinules on the anterior surface along the distal margin of each 2nd exopodal segment from the 2nd to 4th legs. The 5th pair of legs is lacking.

MALE. The total length based on 40 specimens ranges from 2.01 to 2.24 mm. The proportional lengths of the metasome and the urosome are about as 2.4:1. The general shape of the metasome is similar to that of the female, except that in lateral aspect the postero-lateral points are slightly curved downward and, thus, directed backward instead of being directed postero-dorsad as in the female. These points are also smaller than in the female.

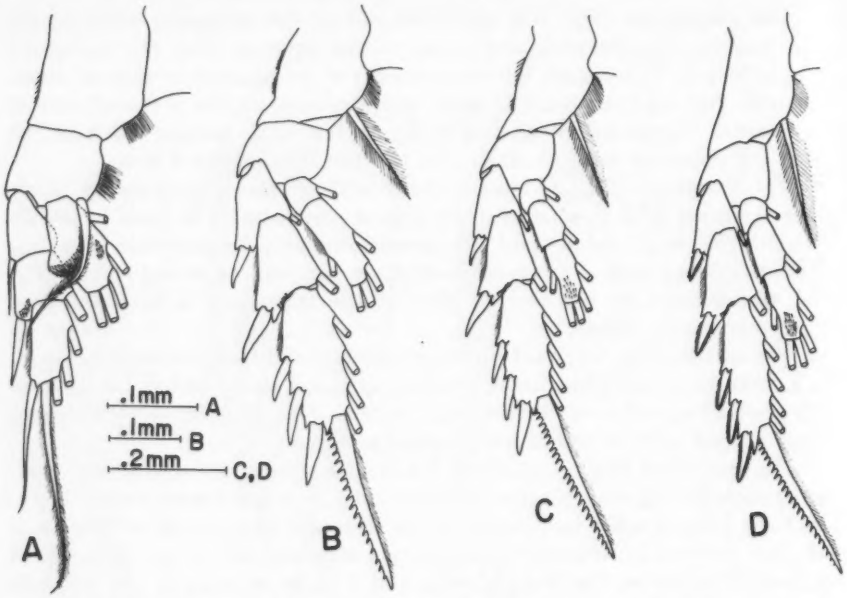

FIG. 2. *Bradyidius saanichi*, n. sp. Female: A, 1st leg; B, 2nd leg; C, 3rd leg; D, 4th leg.

The urosome consists of 5 free segments and the caudal rami. The segments and the caudal rami have the following proportional lengths, from anterior to posterior: 12:27:21:20:8:12. The distal margins of the 2nd to 4th urosomal segments are fringed each with a row of small spinules. The caudal ramus is a little longer than wide (4:3).

The left antennule is 24-segmented as in the female, but the right antennule (Fig. 3C) is 23-segmented, as the 19th and 20th segments are fused together. The arrangement of the setae and aesthetes is, however, the same in both members of the pair.

The antenna (Fig. 3D) is similar to that of the female, except for the following details: The coxa bears a conspicuous row of hairs; the 1st endopodal segment is without setae; and the 2nd endopodal segment carries 7 setae on the inner lobe and 6 setae on the outer lobe, instead of 9 and 7 setae, respectively.

The mouthpart appendages are very reduced as compared with those of the female. The mandible (Fig. 3E) is without a chewing edge. In the maxillule (Fig. 3F) the 1st and 2nd inner lobes are devoid of setae or spines. The maxilla is reduced to a mere small lobe without any setae. The maxilliped (Fig. 3G) is similar to that of the female except that the coxa bears only 2 setae distally.

The 1st to 4th pairs of swimming legs are like those of the female, but the external spines of the exopods of the 1st legs (Fig. 3H) are shorter as

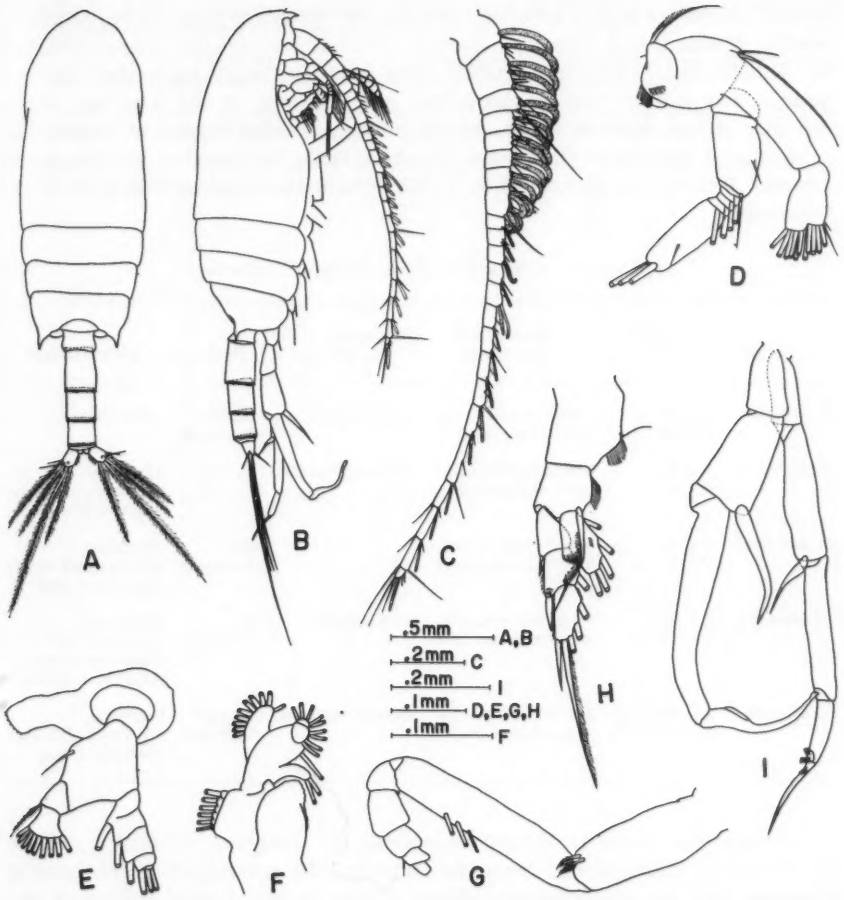

FIG. 3. *Bradyidius saanichi*, n. sp. Male: A, habitus, dorsal view; B, habitus, lateral view; C, right antennule; D, antenna; E, mandible; F, maxillule; G, maxilliped; H, 1st leg; I, 5th pair of legs, anterior aspect.

compared with those of the female. The 5th pair of legs (Fig. 3I) is asymmetrical, although each leg is composed of a basipod, an exopod, and a 1-segmented endopod. The right exopod is 2-segmented, while the left exopod is 3-segmented. There are found 3 patches of hair on the articulation between the 2nd and 3rd exopodal segments of the left leg.

DISCUSSION

Bradyidius tropicus is known to be closely related to *B. armatus*, differing only in size and in the number of teeth on the terminal spines of the exopods

of the 2nd to 4th legs. The other members of the genus seem to be, however, readily distinguished from one another. *Bradyidius angustus* was established by Tanaka (1957) to accommodate some male specimens from the Japanese waters. The male of this species is well characterized by the structure of the 5th pair of legs, both of which are uniramose and about equal in length. The remaining 5 species in the genus, in which both the females and the males are well known, are compared in Table I from information available in the literature.

TABLE I. Comparison of five species of *Bradyidius*.

Species	Length (*mm*)	Metasomal processes	Endopods of 2nd to 4th legs	Rostrum	Male 5th legs
B. armatus	♀ 2.65 ♂ 2.20	reach end of genital segment	with spinules	slightly divergent	uniramose
B. similis	♀ 3.0 ♂ 2.4	reach middle of genital segment	without spinules		biramose; left basipod longer than right one
B. pacificus	♀ 4.4 ♂ 3.3	reach close to end of genital segment		not divergent	biramose; left basipod longer than right one
B. arnoldi	♀ 1.67 ♂ 1.23	reach close to end of genital segment	with spinules	not divergent	biramose; right and left basipods about equal in length
B. saanichi	♀ 2.33–2.56 ♂ 2.01–2.24	reach close to end of genital segment	without spinules	strongly divergent	biramose; left basipod longer than right one

Bradyidius saanichi closely resembles *B. pacificus* (Brodsky, 1950), but is readily distinguished from the latter by the strongly divergent rostral processes and the characteristic shape of the postero-lateral points of the metasome. In addition, *B. saanichi* is smaller in size as compared with *B. pacificus*.

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New Methods for Reducing the Toxicity of Kraft Mill Bleachery Wastes to Young Salmon[1]

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ABSTRACT

Kraft pulp mill bleachery effluents and those from the unbleached screening operation were tested, separately and in combination in the proportions as they would exist in the main effluent of a particular mill, for toxicity to young Atlantic salmon, *Salmo salar*.

The bleachery component from the chlorination stage, tested under neutral *p*H conditions, was considerably more toxic than the other components.

Major reductions in the toxicity of the chlorination stage component were accomplished by treating it with small quantities of sulphur dioxide. Lesser but still appreciable toxicity reductions were observed in the whole effluent when the chlorination component was thus treated before combination with the other components.

The toxicities of laboratory-produced filtrates from certain modified first-stage (chlorination) bleaching procedures were also evaluated. Notably, much less toxic filtrates resulted when substantial mixtures of chlorine dioxide and chlorine were substituted for the normal chlorine dosage in the first bleaching stage.

A number of other treatments, methods, and their relative effectiveness are discussed.

INTRODUCTION

This paper gives the results of a search for ways of reducing the toxicity of wastes from bleached kraft pulp mills. Effluents from such mills, their components, and treatments of the components were evaluated for their relative toxicities to young Atlantic salmon using small-scale laboratory bioassays. The more effective treatments were later retested using large-scale continuous-flow bioassays.

A laboratory bioassay technique using circulating solutions, small test animals, and relatively small quantities of test solutions has been described in Betts et al. (1966). Such a technique is faster and easier than continuous-flow bioassays designed for biological research. In particular, where numerous test solutions are involved, which must be compared with one another, or with a common standard, the use of such screening procedures is desirable.

The kraft bleachery from which the effluents were obtained is a six-stage system: chlorination, caustic extraction, hypochlorite, chlorine dioxide, caustic extraction, chlorine dioxide. The pulp bleached in this operation has been cooked to a permanganate number of approximately 16, and the wood used is a mixture of spruce and fir, with the former species predominating.

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The unbleached screen-room effluent was from the mill producing the above pulp.

The effluents studied were basically three component solutions: bleachery chlorination stage effluent, bleachery first caustic extraction stage effluent, and unbleached screen-room effluent. In the combined effluent the proportions of the three were 1:1:0.11, respectively.

As described later in the section dealing with Results, the initial continuous-flow bioassays clearly established that the combined effluent, even when neutralized, was relatively toxic to young salmon. Also, of the three components, the bleachery chlorination effluent was nearly as toxic as was the combined effluent. Therefore, the work centred mainly around a study of the chlorination effluent, certain first-stage bleaching variations, and various chemical treatments and adjustments of the (standard) first-stage chlorination effluent, using the aforementioned small-scale laboratory bioassays to evaluate the variations.

Since little information was available from the literature, the approach used was largely empirical. It was realized from the first that some of the effluent treatments used offered little promise of an economical solution to the toxicity problem, but it was hoped that they might furnish some insight to the basic mechanisms involved. There was also an urgency to come forward with an answer to a particular effluent toxicity problem.

An attempt to determine the cause of chlorination effluent toxicity to fish was simultaneously begun and is still in progress. Success in this direction would possibly contribute to devising a more elegant method of reducing the toxicity from this source.

MATERIALS AND METHODS

Bioassay Equipment and Procedures

Two types of bioassays were used in this work. A continuous-flow method was used for the more conclusive tests and was usually carried out for 96 hr of exposure. In these tests, a constant flow of the waste or effluent being tested and a constant flow of dilution water was run into each test tank. These two streams mixed in a funnel just above the tank. Total flow was 280 ml/min in each tank. There were four rectangular test tanks each having a capacity of approximately 47 litres.

The desired flow of dilution water was obtained by using a constricted orifice nozzle delivering from a constant head tank. Similar capillary orifices delivered the desired flow of waste to each test tank from a constant head tank. Temperature was controlled by regulated glass-covered heaters in the head tanks. Overflow from the constant head tank of waste was collected and pumped back into the system for economy and smooth operation. This constant-flow apparatus maintained suitably high concentrations of oxygen in the test tanks without need for aeration which would have been undesirable since some of the toxic materials might have been stripped from the test solutions.

The second type of bioassay (Betts et al., 1966) involved the use of smaller equipment, smaller test animals, smaller volumes of test solutions, and a larger

number of test tanks than the apparatus described above. Static tanks of waste were undesirable because small salmon are adapted to conditions of flowing water and also because of the danger of pockets of deoxygenation near the fish which might lead to their death. There was also a need to carry out many tests on various wastes and a number of treatments of the wastes, whereby the use of continuous-flow techniques would be too cumbersome. Consequently, laboratory-sized equipment was designed which featured circulation of water and waste mixtures.

Basically the laboratory-sized equipment consisted of an acclimatizing tank and a series of test tanks. The acclimatizing tank (Fig. 1) was equipped with means of water circulation, temperature control, and aeration. The tank itself was fabricated of Plexiglas,[2] 48 × 16 × 4 inches in overall dimensions, and partitioned into four sections to ensure positive flow over the fish. Water flowed into each section, or trough, from a 30-gal head tank in sufficient quantity so that it moved along the troughs at the rate of 21–24 inches/min. Water issuing from the screened ends of the troughs was collected in another 30-gal plastic tank and pumped back to the head tank. The water was completely replaced once each day with fresh water. Temperature control was achieved by circulating coolants in stainless steel tubes. Aeration was continuous. This equipment supported a stock of 600–4000 young salmon, depending on size, for subsequent testing.

The test apparatus consisted of seven test tanks. These were of moulded polyethylene approximately 20 × 16 × 12 inches in depth (Fig. 2). Each contained a Plexiglas trough (Fig. 3) with approximate dimensions of $17\frac{1}{2} \times 3\frac{1}{4} \times 3$ inches in depth with screened ends, the troughs being partially immersed in the test solutions. Each tank was equipped with a small circulation pump, and all tanks were immersed in a large constant temperature bath. The test solution was pumped from each test tank into one end of the corresponding retention trough at a rate which produced approximately 12 inches of flow per minute along the trough. It then ran through a suitably elevated hole in the end of the trough into the main body of test solution in the tank.

In all tests, 10 fish were used per tank.

Standard Procedures Used for Handling and Testing Effluents

Certain standard handling and testing conditions were chosen to fit the general characteristics of the effluents available and under study. Briefly, these were:

1. Effluents were as fresh as possible. Transportation problems were encountered, but generally effluents were 1–3 days old.
2. Chlorination effluent was adjusted to 450 ppm chemical oxygen demand (C.O.D.) with natural brook water.
3. Caustic extraction effluent was adjusted to 400 ppm C.O.D.

[2]Rhom & Haas Co. registered trademark.

4. Unbleached screen-room effluent was adjusted to 2490 ppm Na_2SO_4 (as might be contributed from residual black liquor).
5. Combined effluent consisted of 1 part chlorination effluent, 1 part caustic extraction effluent, and 0.11 part unbleached screen-room effluent.
6. When tests were run with only one or two of the three components, unpolluted brook water was substituted for the missing portions.
7. All treatments were carried out without prior adjustment of *p*H, temperature, etc., unless this was part of the method of treatment. The effluents were, however, adjusted as shown in 2, 3, and 4 prior to treatment. All calculations were made on the basis of adjusted effluent.
8. After treatment, all effluents tested were adjusted to *p*H 7.0 with calcium hydroxide.
9. A control solution consisting of 75% neutralized, filtered, untreated chlorination effluent was used for comparison in most tests. This is referred to as the "effluent control."
10. Residual chlorine, which occasionally existed in the chlorination effluent upon arrival, was eliminated before the effluent was used. A small quantity (about 1 lb, air-dry basis) of unbleached kraft pulp was added to the effluent and stirred manually until no positive test for residual chlorine was obtained using the standard thiosulphate procedure (Rapson, 1965, p. 376).
11. Test temperatures were maintained at 14 ± 1 C.

Test Effluents

Mill effluents used and treatments prior to testing

The following effluents, treated and untreated, were tested for toxicity to the young salmon.

1. Chlorination effluent, untreated except as described under the Standard Procedures, was used.
2. Combined effluent, untreated except as described under the Standard Procedures, was used.
3. Sulphur dioxide in the following concentrations, based on weight of SO_2 to weight of effluent, was used to treat the chlorination effluent: (a) 1.0%, (b) 0.05%, (c) 0.02%, (d) 0.01%, (e) 0.005%.
4. The chlorination effluent was treated with 0.01% sulphur dioxide and then combined with caustic extraction and screen-room effluents.
5. The combined effluent was treated with 0.01% sulphur dioxide, based on weight of chlorination effluent only.

6. The chlorination effluent was treated with ozone until a lightening in colour was noted. From previous gas-flow measurements, it was calculated that 1.54 g of ozone were used in treating 16.5 litres of effluent.

7. Sodium hydroxide (20% NaOH) was used to raise the chlorination effluent to *p*H 12. The temperature was raised to 50 C; this was followed by rapid cooling, with ice cubes in a polyethylene bag, to 14 C.

8. The same procedure as for test 7 was used except that the effluent was first heated to 50 C and then brought to *p*H 12 with 20% NaOH, followed by cooling.

9. Ascorbic Acid, because of its reducing properties, was used under conditions chemically equivalent to those for test 3c.

10. The same procedure as in the preceding No. 9, except that sodium sulphide was used.

11. The chlorination effluent was neutralized with lime (CaO) and aerated actively for 24 hr.

12. Calcium carbonate ($CaCO_3$) was used to neutralize the chlorination effluent. The *p*H was brought to 6.5 with considerable difficulty, and it was necessary to use lime to make the final adjustment to *p*H 7.0.

13. Chlorine dioxide was added to the chlorination effluent in an amount equivalent to 0.01% SO_2.

VARIATIONS IN THE FIRST-STAGE CHLORINATION

Tests were conducted on the following effluents, prepared under laboratory conditions but representing possible pulp mill bleaching operations:

14. Chlorine dioxide was substituted for the chlorine normally used in the first bleaching stage while other conditions were kept constant (4.50% chlorine on pulp weight ≡ 1.71% chlorine dioxide on pulp weight). The filtrate, after removal of the pulp, was adjusted and tested as previously described under untreated chlorination effluent.

15. A first-stage filtrate was produced as described for test 14, except that the 4.5% Cl_2 normally used was replaced by an equivalent amount of calcium hypochlorite, and the *p*H of the pulp mixture adjusted to 5 to achieve hypochlorous acid conditions.

16. The same procedure as for test 14 was used, except that only 70% of the chlorine dosage (4.5% by weight of pulp) was substituted by an equivalent amount of chlorine dioxide (i.e. 2.63 g Cl_2 ≡ 1 g ClO_2 in oxidizing power).

17. The same procedure as for test 16 was used, except that only 55% of the chlorine dosage was substituted by an equivalent amount of chlorine dioxide.

18. The same procedure as for test 16 was used, except that only 30% of the chlorine dosage was substituted by an equivalent amount of chlorine dioxide.

MISCELLANEOUS TESTS

In the following tests the chlorination effluent was treated in batches or with several concentrations of a particular treating material, and tested at a concentration of 56%. Batch treatments in tests 19 and 20 were employed to provide, if possible, information on the detoxification capacity of the resin or charcoal.

19. Chlorination effluent was passed through a 1 × 28.5-inch cylindrical column of Duolite[3] "3" anion exchange resin in four consecutive 5-litre batches. Each batch was neutralized, filtered, and tested separately.

20. Chlorination effluent was passed through a 1 × 28.5-inch cylindrical column of activated charcoal in four consecutive 5-litre batches. Each batch was neutralized, filtered, and tested separately.

21. Hydrogen peroxide (30% H_2O_2) was added to chlorination effluent in amounts yielding 0.1, 0.08, 0.06, and 0.04 ml H_2O_2 per litre of effluent.

CONTINUOUS FLOW BIOASSAY TESTS

The effectiveness of the sulphur dioxide treatment in reducing the toxicity of the chlorination effluent was subsequently checked by continuous-flow 96-hr bioassay tests. Three tests were conducted as follows:

22. The combined effluent without sulphur dioxide treatment was used as for test 2.

23. The combined effluent was used with treatment of the chlorination effluent by 0.01% sulphur dioxide, as for test 4.

24. The combined effluent was used with treatment of the chlorination effluent by 0.02% sulphur dioxide.

FISH CHARACTERISTICS, CARE AND HANDLING

Newly-hatched Atlantic salmon, *Salmo salar*, obtained from the Dominion Government Fish Hatchery, South Esk, N.B., in early May 1965, were used in all tests. The fish, at an age of 3–4 weeks, were placed in the acclimatizing tank at a temperature of 9 C. The temperature was gradually increased to about 15 C over a period of 8 days, where it was maintained during the experiments. During the period of raising the temperature, and over the subsequent 2 weeks, the mortality was extremely high, although every effort was made to follow hatchery procedures. A number of conditions for handling fish were established during this period, as follows:

[3]Diamond Alkali Co. registered trademark.

The water supply was changed daily. Water was obtained from an unpolluted source known as Christopher Brook. The flow of water in this brook was estimated at approximately 8000–10,000 gal/min during average summer conditions. Total hardness of this water was about 90 ppm $CaCO_3$ and the *p*H about 7.8.

No metal of any kind (e.g. laboratory tubing clamps) other than stainless steel was allowed to contact the water. The fish were fed fresh chopped and screened calves liver three times a day in small portions. Except for feeding times and periodic checks, the tank was kept covered with a wooden cover. Dead fish were removed from the tank as soon as possible.

At the conclusion of the tests in late September, the fish had increased to a size of 2–2½ inches in length, and at this time, the acclimatizing tank would hold a maximum of around 600 fish as compared with the initial 4000.

The dissolved oxygen content of the water in the acclimatizing tank was maintained at 8–11 ppm by artificial aeration.

Throughout the testing period, the mortality of the fish during holding was less than 5% and usually less than 1% per day. There was no evidence of any major changes in fish response with increasing size and age.

Interpretation of Data

Fish response data obtained from the tests are presented in the form of "time to 50% mortality" (also referred to as median resistance time) or "per cent dead in 48 hr" at each concentration level or test point. This is an alternative method to the usual method of reporting bioassay results on the basis of "median tolerance limit" (TLM) for a given time-period.

To obtain the time to 50% mortality, points representing time (log scale) versus per cent mortality (probability scale) were plotted on log-probability paper (e.g. K & E 46 8080). A straight line was drawn through the points, attaching the most significance to those points closest to the median mortality. In many cases, it was necessary to draw the line through only the points immediately above and below 50% mortality. By taking the perpendicular from the intersection of the plotted line with the 50% mortality line on the graph, the time to 50% mortality was determined from the time scale.

Comparison between effluents, treatments, etc., on the basis of the time to 50% mortality, were made possible by using the results from the control effluent as a common reference point. By using a test effluent in most of the tests at the same concentration as the control effluent (75%), a basis for comparison was established.

The control effluent was very useful, not only as a reference point, but also to detect variations in effluent toxicity, which could not otherwise be monitored, since the toxic material is as yet unidentified. Also, variable fish response could be detected. Apparent differences in response due to variation in effluent toxicity were noted in tests 3a, 3d, and 18. The toxicity of the effluent appeared lower than normal (higher time to 50% mortality) in these, and the opposite was noted in tests 2 and 4.

The concentration of the control effluent was selected for its ability to cause 100% mortality in a short time, usually less than 10 hr; hence the effluent control and water control are at opposite ends of the scale of toxicity, with the test effluents falling in between.

RESULTS

Initial Continuous-Flow Tests

These tests showed that the chlorination effluent is considerably more toxic than either the caustic extraction or unbleached screen-room effluent. The chlorination effluent was indicated to be also more toxic than the combined effluent, although this was not supported in tests 1 and 2.

Circulation Tests

INEFFECTIVE TREATMENTS

Briefly, it can be said that ineffective treatments (Table III, tests 6, 9, 10, 12, and 13) showed up rather obviously, and there was little difficulty in judging such results. Test 13 (chlorination effluent treated with chlorine dioxide) revealed a rather surprising increase in the toxicity. In view of later tests where chlorine–chlorine dioxide mixtures had been used to bleach the pulp, the cause of this toxicity remains to be determined. It was surprising that ozone treatment (test 6) did not decrease toxicity because it was obvious that some oxidation had occurred. Ascorbic acid (test 9), sodium sulphide (test 10), and calcium carbonate (test 12) appeared to be ineffective treatments.

EFFECTIVE TREATMENTS

These tests (Table II–IV) indicated that several treatments of the chlorination effluent reduced its toxicity appreciably: 1. Sulphur dioxide (tests 3, 4, and 5). 2. Making effluent alkaline followed by heating to 50 C (test 7). 3. Heating followed by raising the *p*H (test 8). 4. Aeration (test 11). 5. Anion exchange treatment (test 19). 6. Activated charcoal treatment (test 20).

In addition, the substitution of chlorine dioxide for all or a large part of the chlorine normally used in the first stage of bleaching produced a less toxic effluent (tests 14, 16, and 17). First-stage bleaching conditions favouring the presence of hypochlorous acid also produced less toxic filtrates (test 15).

Final Continuous-Flow Tests

The final 96-hr continuous-flow bioassays (Table V), carried out on combined effluents, confirmed that treatment of the chlorination component with 0.01% SO_2 (test 23), effects an appreciable reduction in toxicity compared with no treatment (as in test 22), particularly at the 18% and the 32% effluent concentrations. Increasing the SO_2 treatment dosage from 0.01 to 0.02% resulted in a further but smaller reduction in toxicity (test 24).

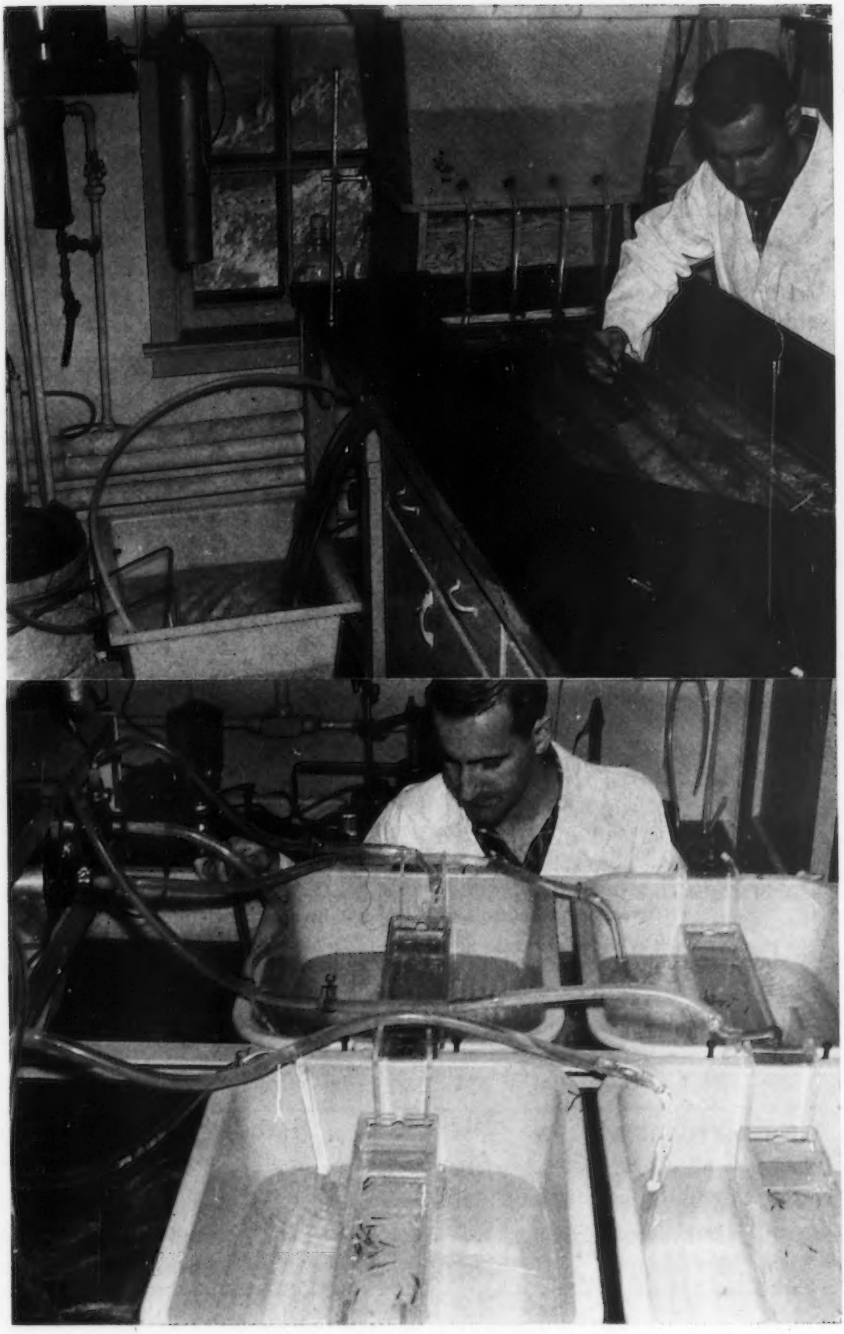

FIG. 1. *Top.* Acclimatization tank and associated head tank and sump tank. One of two cooling units shown at lower left.

FIG. 2. *Bottom.* Four of the seven test tanks immersed in large temperature-controlled bath. Plexiglas fish-retention troughs partly submerged in test tanks.

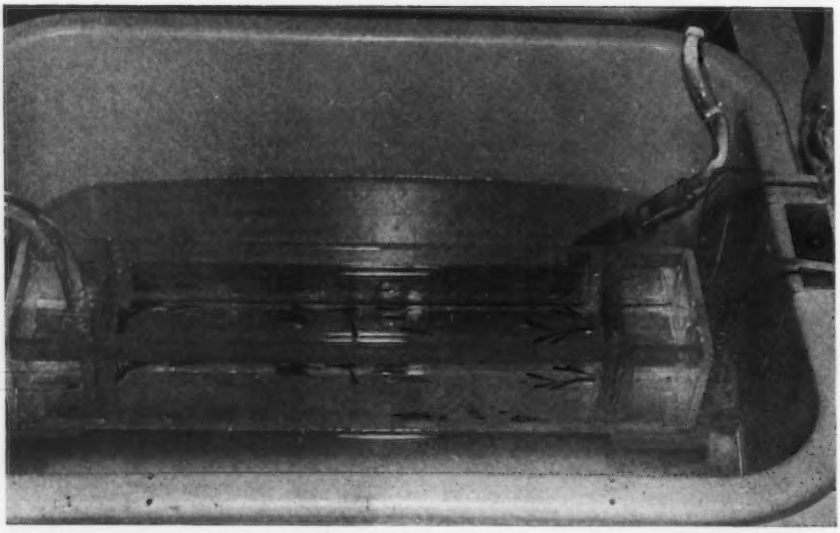

FIG. 3 Plexiglas fish-retention trough showing inlet at left and outlet at right. Fish-retaining screens are mounted several inches in from each end.

CONCLUSIONS

Items 2, 3, 5, and 6 of the "Effective Treatments" discussed above, are considered to be unattractive economically, and therefore do not seem to warrant further discussion at this time. The capacity of charcoal to detoxify the effluent (test 20) was not determined.

Aeration was effective in the experiments (test 11) and is apparently economically feasible. However, it was found to be much less effective in previous continuous-flow bioassay tests of 96 hr duration which are not dealt with in this paper. This method of reducing toxicity, therefore, deserves further consideration and investigation before a positive conclusion can be made.

The use of hypochlorous acid first-stage bleaching conditions is well known to be undesirable from a pulp quality standpoint. However, it seems appropriate to note that the resulting filtrates (test 15) appear to be less toxic than those of common chlorination procedures.

The use of chlorine–chlorine dioxide mixtures in the first stage of a multistage kraft bleaching sequence is certainly an attractive alternative to present practices from both pulp quality and effluent toxicity standpoints as shown by tests 16 and 17. It is well known that this may become economically feasible in the near future (Rapson, 1965; Rapson and Anderson, 1966; Singh and Andrews, 1965).

Sulphur Dioxide

From the test results (test 3c and 3d), it is evident that treatment of the chlorination effluent with small amounts of sulphur dioxide is an effective method of reducing toxicity. However, when the treated chlorination effluent is combined with the caustic extraction and unbleached screen-room components and tested, the benefits of SO_2 treatment are not as great (test 4). The reason for this has not yet been determined, although there may be other factors in addition to the known low toxicity of the screen-room and caustic extraction effluents (Table I) which affect the relative toxicity level when they

Table I. Hours to 50% mortality of young salmon (or per cent dead in 96 hr) for various concentrations of the combined and component effluents, tested by 96-hr continuous-flow bioassays.

Effluent tested	Temperature (C)	Effluent concentration				Water control
		32%	18%	10%	5.6%	
Combined effluent	16	10[a]	20	(0%)	(0%)	(0%)
Combined effluent	11	22	(30%)	(0%)	(0%)	(0%)
Chlorination effluent	11	13	56	(0%)	(10%)	(0%)
Kraft screen-room effluent	16	50	(0%)	(0%)	(0%)	(0%)
Caustic extraction effluent	16	(30%)	(0%)	(0%)	(0%)	(0%)

[a]Ten fish tested at each concentration.

TABLE II. Hours to 50% mortality of young salmon (or per cent dead in 48 hr) for various concentrations of treated and untreated effluents, tested by 48-hr circulating bioassays.

	Effluent concentration					Control	
Test	75%	56%	32%	18%	10%	Water	Effluent 75%
1	4.4[a]	6.4	20.9	(0%)	none used	(0%)	4.4
2	1.9	2.5	3.6	(40%)	(0%)	(0%)	2.6
3a	not tested	(20%)	(0%)	(0%)	(0%)	(0%)	10.3
3b	not tested	100% dead in 1st hour	(0%)	(0%)	(0%)	(0%)	7.5
3c	(20%)	(10%)	(0%)	(0%)	(0%)	(0%)	8.2
3d	(0%)	(0%)	(0%)	(0%)	(10%)	(0%)	10.9
3e	13.3	17.2	(0%)	(0%)	(0%)	(0%)	5.7
4	10.6	13.8	(0%)	(0%)	(0%)	(10%)	3.35
5	16.0	17.0	(0%)	(0%)	(10%)	(0%)	4.1

[a]Ten fish tested at each concentration.

TABLE III. Hours to 50% mortality of young salmon (or per cent dead in 48 hr) for various concentrations of treated or modified effluents, tested by 48-hr circulating bioassays.

	Effluent concentration					Controls	
Test	75%	56%	32%	18%	10%	Water	Effluent
6	5.9[a]	7.0	17	(20%)	(10%)	(0%)	5.4
7	(10%)	(0%)	(10%)	(0%)	(30%)	(0%)	none used
8	(0%)	(20%)	(0%)	(0%)	(0%)	(0%)	5.7
9	4.6	5.9	(40%)	(0%)	none used	(0%)	3.9
10	4.3	3.8	5.6	13.5	(10%)	(0%)	7.2
11	none used	(10%)	(0%)	none used	(0%)	(0%)	4.5
12	none used	7.2	18.5	none used	(0%)	(0%)	6.6
13	2.4	3.1	8.0	(0%)	(0%)	(0%)	3.7
14	(10%)	(0%)	(0%)	none used	(0%)	(20%)	none used
15	(0%)	(0%)	(0%)	none used	(0%)	(0%)	none used
16	none used	(0%)	(0%)	none used	(0%)	(0%)	none used
17	(10%)	(0%)	(0%)	(0%)	(0%)	(0%)	4.6
18	11.4	24.7	(0%)	(0%)	(0%)	(0%)	11.4

[a]Ten fish tested at each concentration.

TABLE IV. Hours to 50% mortality of young salmon (or per cent dead in 48 hr) for the chlorination effluent batch-treated to determine detoxification capacity or effective concentration of treating agent. Tested by 48-hr circulating bioassays at 56% concentration.

Test	Batch, treatment or concentration				Controls		
					Water	Effluent 75%	Effluent 56%
19	1st batch[a] 47.0	2nd batch 14.8	3rd batch 13.0	4th batch (20%)	(0%)	6.7	8.5
20	1st batch (0%)	2nd batch (0%)	3rd batch (0%)	4th batch (0%)	(0%)	4.4	6.5
21	0.1 ml/litre 13.8	0.08 ml/litre 10.4	0.06 ml/litre 18.9	0.04 ml/litre 7.4	(0%)	3.8	5.7

[a]Ten fish tested.

TABLE V. Hours to 50% mortality of young salmon (or per cent dead in 96 hr) for various concentrations of the combined effluent after sulphur dioxide treatments of the chlorination effluent, tested by 96-hr continuous-flow bioassays.

Test	Effluent concentration				Water control
	65%	32%	18%	10%	
22	4.0[a]	9.5	22.0	(10%)	(0%)
23	6.7	27.5	(0%)	(0%)	(0%)
24	8.4	46.5	(0%)	(0%)	(0%)

[a]Ten fish tested at each concentration.

are combined. For example, when all components are present, concentrations of inorganic and organic material are increased. Treatment of the combined effluent with SO_2 (test 5) appears to yield results similar to those of test 4.

The final 96-hr continuous-flow bioassays on combined effluent (Table V) showed that, at 18% untreated effluent concentration, 50% of the test fish died in 22 hr (test 22). Treatment of the chlorination effluent component with 0.01% sulphur dioxide reduced the toxicity to a point where none of the test animals died in 96 hr (test 23).

COST OF SULPHUR DIOXIDE TREATMENT

The cost of applying this treatment to the chlorination effluent would depend, it is believed, on the amount of water being sewered per ton of pulp.

With the chlorination effluent used in these tests, the amount of water sewered at this stage varies between 10,000 and 15,000 US gal/ton.

If sulphur dioxide costs 2.5 cents/lb and the treatment amounts to 0.01% by weight of the chlorination effluent, then the cost per ton at 15,000 US gal/ton of pulp on this basis would be:

$15{,}000 \times 8.34 \times 0.0001 \times 2.5 = 31.2$ cents per unbleached ton.

Methods of Choice

In summary, it may be said that treatment of the chlorination effluent with sulphur dioxide offers a relatively cheap and effective method of reducing the toxicity of kraft bleachery chlorination stage effluents. This method is thus considered to be a "method of choice," although its effectiveness is lessened by combining other effluents with it. Nonetheless, consideration will undoubtedly be given to it above other methods.

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Variability of Oceanographic Conditions Between Ocean Station P and Swiftsure Bank off the Pacific Coast of Canada[1]

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ABSTRACT

Oceanographic observations taken along the line between Ocean Station P (50°N, 145°W) and Swiftsure Bank (48°32′N, 125°00′W) from January 1959 through January 1962 showed that the annual variation of temperature was determined primarily by the heat exchange across the surface and only secondarily by currents. Salinity variations were related to freshwater runoff from land and the component of Ekman transport normal to the coast. Variations of depths of isopycnal surfaces appeared to be related to the curl of wind stress, except near the coast. However, the observed vertical movement of isopycnal surfaces was much larger than that calculated from the wind stress. Near the coast, the vertical movement was related to the component of Ekman transport normal to the coast.

The average total transport through the section, as calculated from the curl of the wind stress, was 5.8 million metric tons per second and was directed northward. The average baroclinic flow was 13 million metric tons per second. These transports were consistent if the mean depth of no motion was at 1400 m with southward flow in the bottom portion.

INTRODUCTION

In April 1959, the Pacific Oceanographic Group of the Fisheries Research Board of Canada, with the cooperation of the Marine Services Branch of the Department of Transport of Canada, initiated a program of serial temperature and salinity observations along a line from Swiftsure Bank (48°32′N, 125°00′W) to Ocean Station P (50°N, 145°W)[3] (Fig. 1) a distance of about 1460 km (788 nautical miles). Initially, seven stations spaced about 200 km apart were occupied at intervals of approximately 6 weeks by the weathership C.C.G.S. *St. Catharines* while en route to and from Ocean Station P. The temperature and salinity of the water were determined at standard intervals to a depth of 2000 m, weather permitting. In addition, bathythermograph observations were made and continuous (thermograph) records of the ship's water intake temperatures were taken. Oceanographic observations at Station P, obtained during alternate 6-week periods, were available from August 1956; additional observations were available from various *North Pacific* and *coastal* cruises from 1959 to 1961.

Preliminary analysis of the data collected during 1959 and early 1960 showed that the greatest variations of the oceanographic conditions occurred in the eastern half of the Line. Therefore, in May 1960, after 10 sections had

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[3]Hereafter referred to as the "Line."

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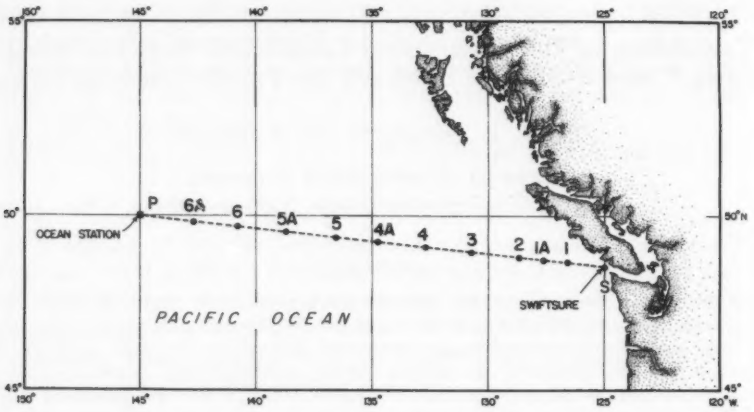


FIG. 1. Station positions along the Station P–Swiftsure line. Stations labelled "A" are supplementary and were not usually occupied by the weathership.

been obtained, the station positions were changed to decrease the spacing in this region. The final station positions are shown in Fig. 1. The number of stations occupied by the weathership could not be increased to more than seven because of the limited time available in its schedule. Therefore, to supplement the weathership data, cruise plans for the *Coastal* and *North Pacific* oceanic surveys were modified to include stations along the Line. In these supplementary sections, dissolved oxygen content was measured and observations were made to the bottom. Also, additional stations, designated by the letter "A" following the station number in Fig. 1, were occupied.

A total of 35 sets of observations (Table I) were made from January 1959 through January 1962. These data form the basis for the present discussion of the variations of temperature, salinity, and density along the Line, and the geostrophic currents and transports across the Line. The sets of observations along the Line are not of sufficient density in either time or space to follow the details of such changes that occur. Observations at Station P (Tabata, MS, 1964) and at anchor stations on the continental shelf (Lane, 1962) show that appreciable fluctuations occur during tidal and shorter periods. Nevertheless, the data demonstrate that systematic changes occur at scales considerably larger than the spacing between stations and the time intervals between cruises

BATHYMETRY OF NORTHEAST PACIFIC OCEAN

The general bathymetry of the northeast Pacific Ocean is characterized by a deep trench (with depths exceeding 5000 m) to the south of the Aleutian Islands and a number of ranges of seamounts rising from the abyssal plain (at depths between 3000 and 5000 m) to the east of 155°W long. Several

TABLE I. List of oceanographic data used.

Cruise No.	Cruise[a] designation	Date	No. of stations	MS Rep. Ser. No.[b]
		1959		
1	NP-59-1	Jan. 20–Jan. 22	6	43
	P-59-1	Jan. 25	1	59
2	P-59-2	Apr. 14–Apr. 18	7	59
3	P-59-2	May 29–May 31	7	59
4	P-59-3	July 8–July 11	7	59
5	P-59-3	Aug. 20–Aug. 23	7	59
6	P-59-4	Sept. 23–Sept. 26	7	59
7	P-59-4	Oct. 27–Nov. 2	5	59
8	P-59-5	Dec. 9–Dec. 18	3	98
		1960		
9	P-59-5	Jan. 19–Jan. 26	7	98
10	NP-60-1	Feb. 4–Feb. 9	3	63
11	P-60-1	Mar. 2–Mar. 5	5	98
12	P-60-1	Apr. 13–Apr. 17	3	98
13	P-60-2	May 25–May 28	6	98
14	P-60-2	June 29–July 4	7	98
15	P-60-3	Aug. 3–Aug. 6	7	98
16	NP-60-2	Aug. 9–Aug. 12	11[c]	82
	P-60-3	Aug. 10	1	98
17	P-60-3	Sept. 15–Sept. 19	6	98
18	P-60-4	Oct. 26–Nov. 1	2	98
19	P-60-4	Dec. 1–Dec. 11	4	98
		1961		
20	P-61-1	Jan. 18–Jan. 24	6	106
21	LP-61-1	Feb. 6–Feb. 9	9[c]	91
	P-61-1	Feb. 12	1	106
22	P-61-1	Mar. 2–Mar. 6	7	106
23	MR-61-1	Mar. 21–Mar. 24	10[c]	96
24	MR-61-1	Mar. 24–Mar. 27	8[c]	94
25	P-61-2	Apr. 12–Apr. 15	5	106
26	NP-61-1	May 16–May 19	11[c]	101
	P-61-2	May 14	1	106
27	P-61-2	May 23–May 29	8	106
28	P-61-3	June 28–July 1	7	106
29	P-61-3	Aug. 3–Aug. 6	8	106
30	P-61-4	Sept. 13–Sept. 17	8	125
31	MR-61-3	Sept. 25–Sept. 29	8[c]	111
	P-61-4	Sept. 26	1	125
32	P-61-4	Oct. 27–Oct. 30	7	125
33	MR-61-4	Nov. 7–Nov. 11	9	111
34	P-61-5	Dec. 6–Dec. 9	8	125
		1962		
35	P-61-5	Jan. 17–Jan. 22	6	125

[a]P — weathership cruises; NP — North Pacific cruises; LP and MR — coastal cruises.
[b]Fisheries Research Board of Canada, Pacific Oceanographic Group, Manuscript Report Series (Oceanographic and Limnological).
[c]Observations below 2000 m depth taken.

ranges of seamounts oriented roughly parallel to the Pacific coast of Canada terminate near the Line (Fig. 2).

Four large seamounts and six smaller ones lie within 120 km north of the Line, while only one large and three smaller seamounts lie within the same distance to the south (Fig. 3a). The minimum and maximum depths within 30 km of the Line were taken from nautical charts and are intended to show the variability rather than the detailed shape of the bottom. Most of the features shown in Fig. 2 and 3a can be identified in the profile (Fig. 3b) obtained during a soundings run made by the C.N.A.V. *Oshawa* in May 1962.

The mean depth over the Line, including the continental shelf, is 3100 m and the cross-sectional area about 44.8×10^{12} cm^2. The mean depth over the western half of the Line is 3800 m with the bottom sloping upward slightly to the east. In the eastern half, the topography is more rugged and the upward slope more pronounced. The mean depth is 2800 m to the foot of the continental slope and 2400 m including the shelf. The depth decreases from 3500 m at Station 4A to 2500 m at the foot of the continental slope. The continental slope off Juan de Fuca Strait is about 50 km wide. However, the Line crosses the slope obliquely, resulting in a width of 100 km along the Line.

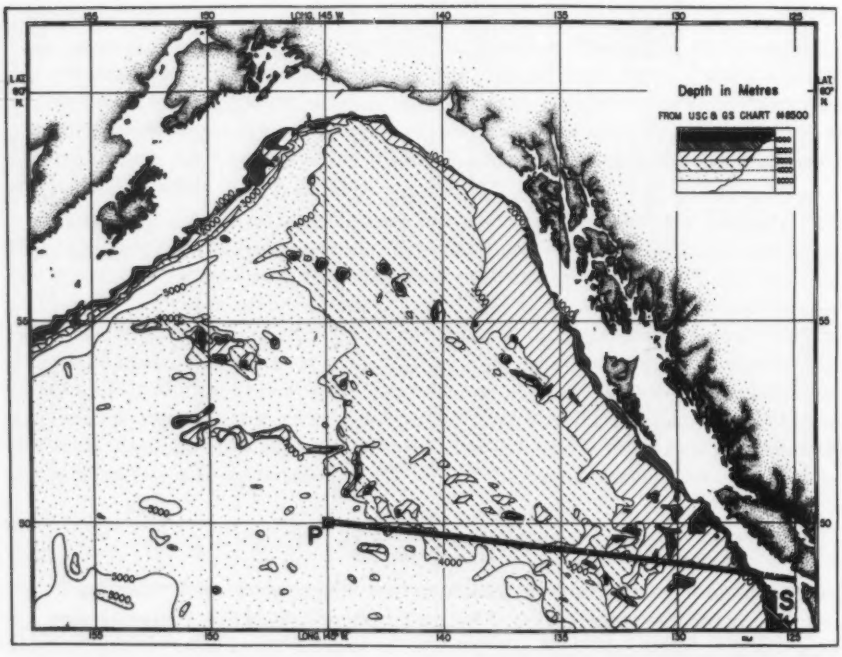


FIG. 2. Bathymetry of northeast Pacific Ocean. The Line is indicated by the heavy line.

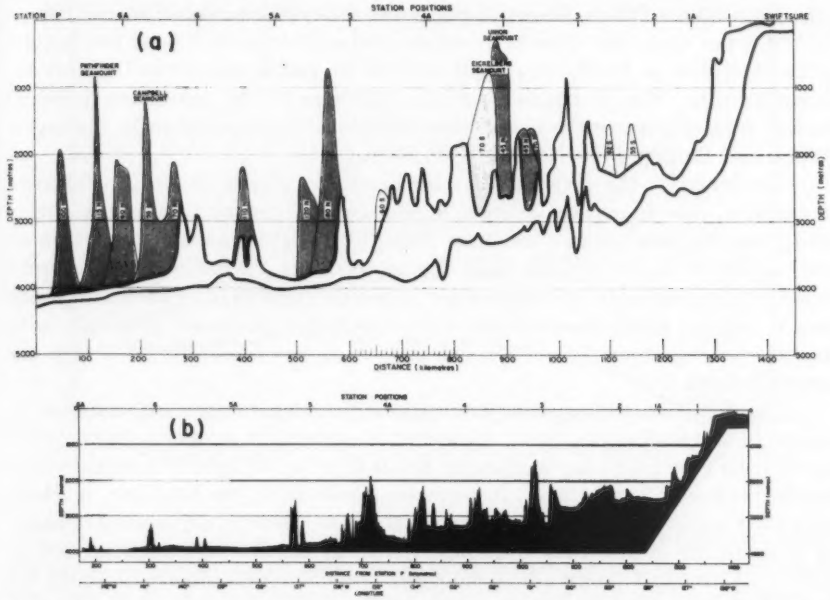


FIG. 3. Bathymetry along the Line. (a) Bottom topography as indicated by the minimum and maximum depths within 30 km of the Line (based on U.S.C.G.S. Chart No. 8500). Seamounts within 120 km of the Line are shown as shaded peaks with distances north or south indicated in kilometres. (b) Bottom profile along the Line based on uncorrected sonic soundings obtained with an Edo echo-sounder and a precision depth recorder. The true depths are slightly greater than shown (about 50 m at 4000 m).

GENERAL OCEANOGRAPHIC FEATURES OF THE NORTHEAST PACIFIC OCEAN

The first systematic study in the vicinity of the Line was made by Doe (1955) on the basis of four oceanographic surveys (1950–52). Since the summer of 1955, a number of oceanographic surveys have been made in the northeast Pacific Ocean including the region containing the Line, and their data have been used to provide an oceanographic description of the subarctic Pacific region (Dodimead, MS, 1958; Bennett, 1959; Uda, 1963; Dodimead et al., 1963 — the last two papers describe the entire subarctic Pacific region).

The main feature of the water in the eastern subarctic Pacific region is the presence of three distinct layers, or zones, in the vertical structure: the upper zone, halocline, and lower zone (Doe, 1955; Tabata, 1960; Dodimead, 1961; Dodimead et al., 1963). The upper zone, about 100 m in depth, contains the major seasonal variations and is the layer that is directly influenced by surface processes.

The upper zone is characterized by low salinities ($32.7 \pm 0.3‰$) and homogeneous structure during the winter months. The halocline forms the transition between the upper and the lower zone. The change of salinity within it is about

1‰, from about 32.8 to about 33.8‰, over a depth interval of about 100 m. In the lower zone, the salinity increases gradually from 33.8‰ at the bottom of the halocline to 34.4‰ at a depth of 1000 m, and finally to 34.7‰ near the ocean bottom. This distinctive salinity structure in the upper few hundred metres is attributed to the excess precipitation over evaporation in the region (Tully and Barber, 1960; Tabata, 1961, MS, 1964).

The water in the upper zone is isothermal in winter (6 ± 3 C). However, in summer, due to surface heating, a well-defined thermocline occurs within which the temperature may decrease (from 13 ± 5 C) by as much as 8 C in an interval of 20 m. In the halocline the temperature generally decreases with depth, but occasionally a temperature inversion (temperature increasing with depth) occurs. In the lower zone, the temperature decreases gradually with depth, reaching about 3 C at a depth of 1000 m and finally to 1.5 C near the ocean bottom.

The dissolved oxygen content in the upper zone is generally constant in winter (0.63 ± 0.05 mg-at/litre). However, in summer it increases slightly with depth from 0.56 ± 0.07 mg-at/litre by about 0.05 mg-at/litre to a maximum at depths between 50 and 100 m. It decreases markedly in the halocline, reaching about 0.3 mg-at/litre at the bottom. In the lower zone it decreases gradually to a minimum of less than 0.05 mg-at/litre at depths varying from 200 to 1200 m. From this minimum it increases gradually with depth to about 0.3 mg-at/litre near the ocean bottom.

The annual variations of temperature, salinity, and dissolved oxygen content of the water at Station P have been described in detail, and the factors governing these changes discussed (Tabata, 1961, MS, 1964). In the upper zone the temperature reaches a minimum in late winter and a maximum in late summer. The annual range of the temperature in the upper mixed layer in the upper zone is about 8 C degrees here and it appears that the ranges in the other parts of the subarctic region are similar to that at the Station (Dodimead et al., 1963). At Station P this range is due primarily to the heat flux across the air–sea boundary; in the other areas of this region the annual variations are likely to be due to heat flux also. The salinity in the upper mixed layer varies from a minimum in late summer to a maximum in late winter. Its annual range is small (about 0.2‰). The surface dilution from excess precipitation over evaporation and the relative ineffectiveness of vertical mixing in the summer months permit the water in this layer to become dilute. However, due to intense vertical mixing (autumnal breakdown of the properties in the upper zone), the upper mixed layer becomes deeper and more saline with time during autumn through winter. The dissolved oxygen content in the upper mixed layer varies from a minimum in late summer to a maximum in late winter. Its annual range is about 0.1 mg-at/litre and is governed chiefly by the annual change of the solubility of oxygen in the water (Tabata, 1961, MS, 1964).

Within the Subarctic region, Dodimead et al. (1963) defined the domains as is shown in Fig. 4. Station P lies in the central subarctic domain and the Line crosses the coastal and the northern tip of the transitional domains.

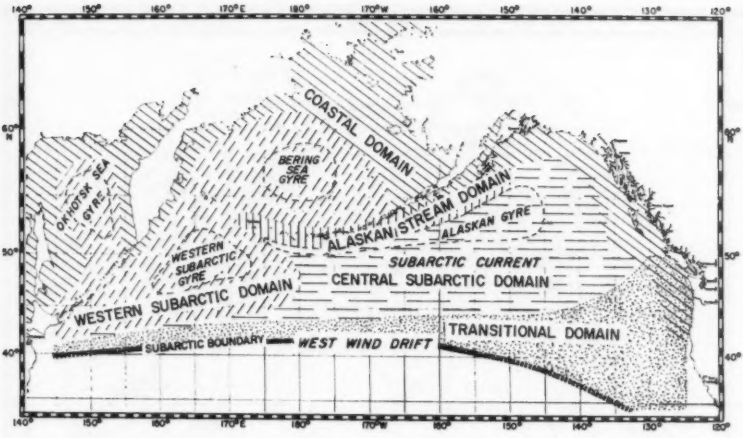


FIG. 4. Schematic diagram of the upper zone domains in the subarctic Pacific (from Dodimead et al., 1963).

To the northwest of Station P lies the "dome" (so-called because the isometers of all properties of water on sections drawn through the cold centre of the Gulf of Alaska are dome-shaped). It is characterized by having water of relatively higher salinity (Fig. 5) and lower temperature (Fig. 6) than is present in the surroundings. Although obscured in surface distributions, the dissolved oxygen content is lower in the subsurface layers at the dome than at corresponding depths in its neighbourhood. From these extreme values the properties of the water generally change gradually to the south and toward the coast. At the surface and coastward from Station P the temperature increases and the salinity decreases along the Line. The dissolved oxygen content generally decreases along the Line.

A comparison of the dynamic height anomaly in the northeast Pacific Ocean (Fig. 7) with the distributions of salinity (Fig. 5) and temperature (Fig. 6) indicates that the "dome" lies in the centre of the cyclonic circulation system of the Alaskan Gyre.

Previously, the major eastward-flowing surface current was termed the "West-Wind Drift" or the "Subarctic Current" (Sverdrup et al., 1942). Recent studies (Dodimead et al., 1963) have indicated that it is appropriate to divide this current into two: the Subarctic Current, the northern eastward flow, and the West Wind Drift, the southern eastward flow (Fig. 8).

The Subarctic Current is apparently the part of the Oyashio that does not actively mix with the Kuroshio and flows eastward, then into the Gulf of Alaska and around the Alaskan Gyre as the westward-flowing Alaskan Stream; it eventually returns to the Oyashio region, thus completing the subarctic circulation system. The West Wind Drift, on the other hand, is the mixture of the Oyashio and the Kuroshio and flows eastward until it approaches the coast

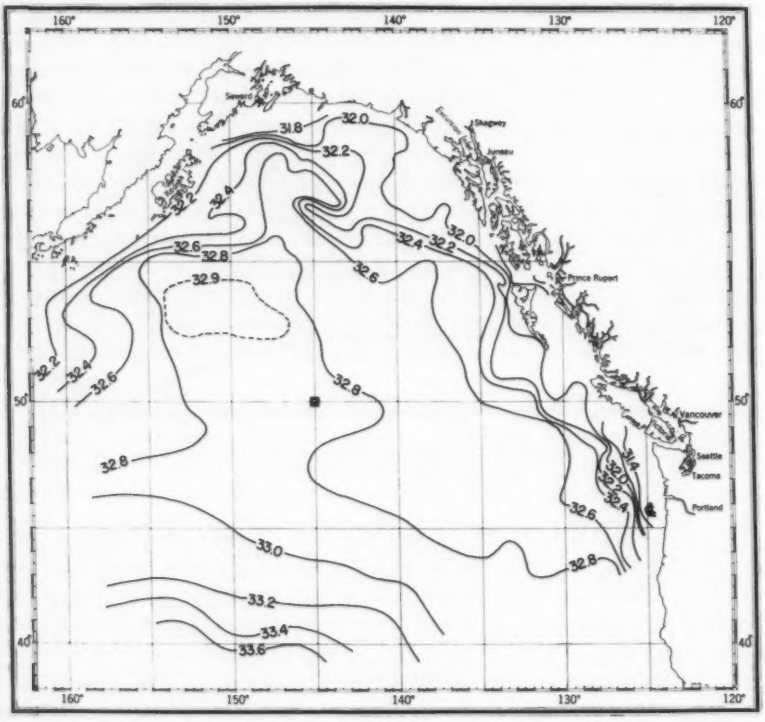


FIG. 5. An example of salinity (‰) distribution at 10 m depth in the northeast Pacific Ocean during May–June 1961.

of North America (about 500 km west of the Washington–Oregon coast). Here it divides to form the southward-flowing California Current and the northward-flowing Alaskan Current. Station P lies in the path of the Subarctic Current, and the Line in the regions of both the Subarctic Current and the West Wind Drift. However, in the vicinity of Station P these two currents are not easily distinguishable.

The coastal region off the Pacific coast of Canada examined by Doe (1955) includes the surface waters that are appreciably diluted by drainage from land. He noted that in summer, following a large land drainage due to melting of snow in the mainland mountains, the freshwater influence is at a maximum and extends farther offshore than in the winter. Barber (1957) showed that during the winter under influence of strong southeast winds, light surface waters accumulate along the coast, displacing the deeper waters offshore. Lane (1962), upon examination of more recent data, also arrived at the same conclusions, that: during summer under the influence of northwesterly winds a divergent situation occurs whereby the surface waters are transported offshore

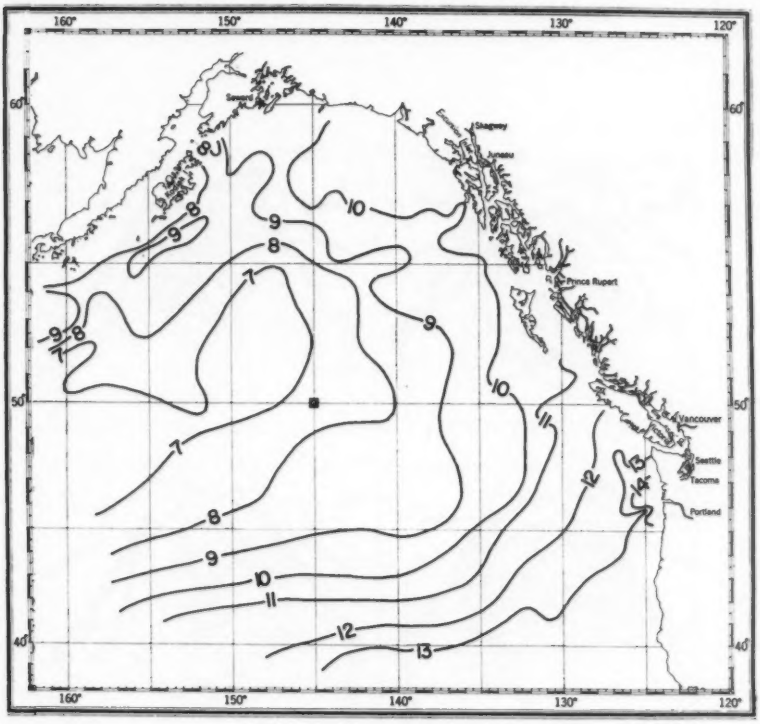


FIG. 6. An example of the temperature (C) distribution at 10 m depth in the northeast Pacific Ocean during May–June 1961. Temperatures were reduced to one time by assuming the rate of change to be the same as observed at Station P during the 6-week cruise.

and the subsurface waters onshore, resulting in local upwelling along the coast; during winter under the influence of southeasterly winds a convergent situation occurs whereby the surface waters accumulate along the coast. The upwelling during the summer had been considered as the agent responsible for the occurrence of cold water adjacent to the coast. The essential result of these processes had been stated earlier by Tully (1938), however.

The western limit of this coastal region was designated by the 32.50‰ surface salinity isopleth (Doe, 1955). Within the coastal region, the salinity increased with depth in the surface zone, whereas in the offshore region, it tended to be constant with depth to the halocline, although in the summer months there occurred a small vertical salinity gradient within the upper zone. Lane (1962) has further remarked that in the coastal region, the upper zone is characterized by a shallow estuarine structure.

Doe (1955) found that the currents in both the coastal and oceanic regions were weak and variable, but persistently northward with a number of eddies

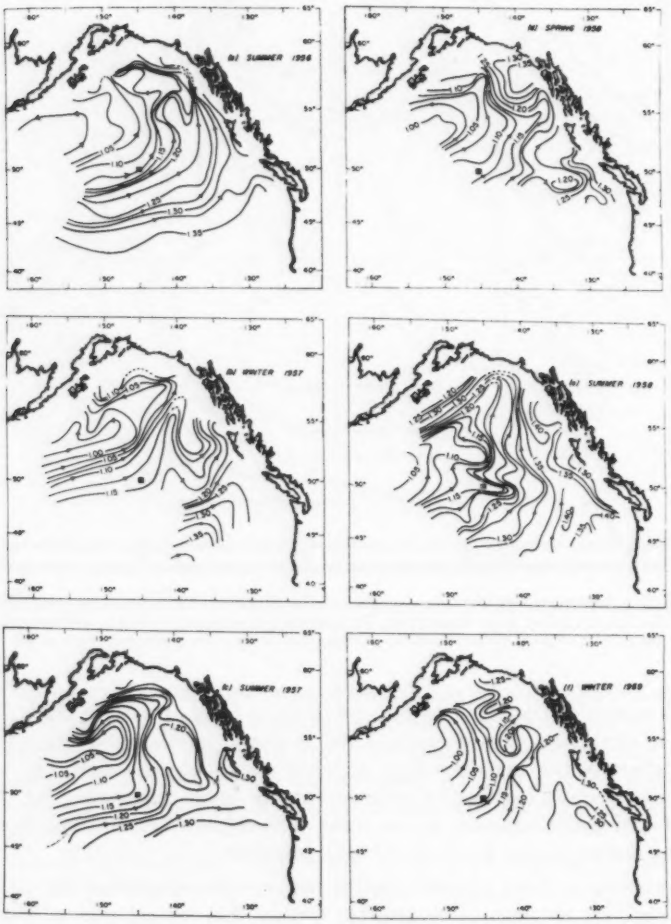
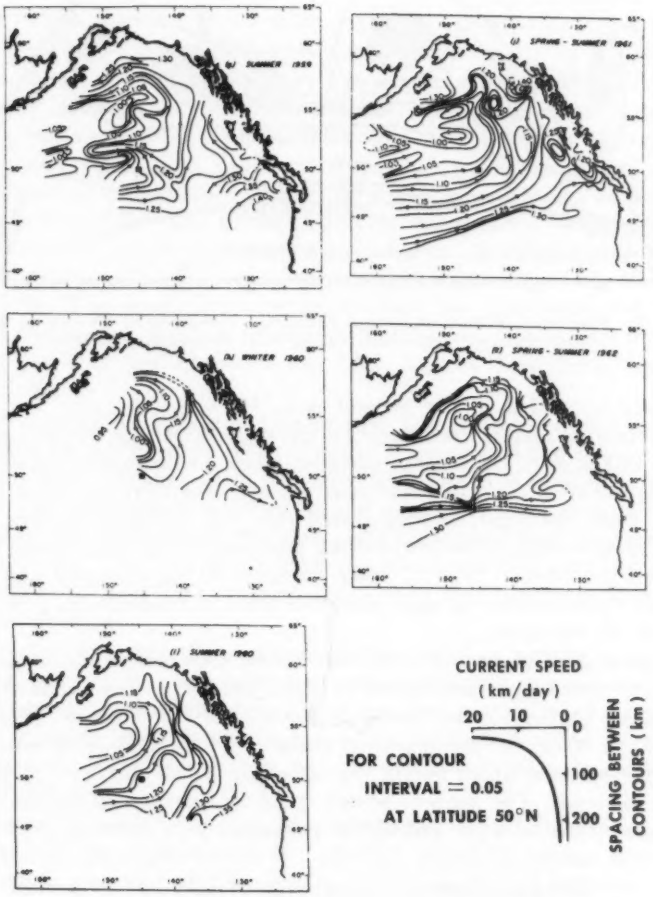


FIG. 7. Distribution of dynamic height anomaly at the surface relative to



1000-m surface (0/1000 decibars) in the northeast Pacific Ocean, 1956–62.

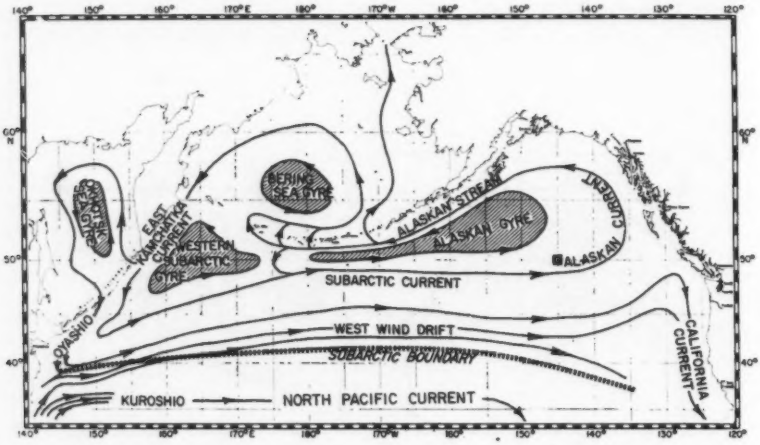


FIG. 8. Schematic diagram of surface circulation (geostrophic) relative to that at 1000-m surface, North Pacific Ocean (from Dodimead et al., 1963).

apparently imbedded in the general flow. He suggested that the flow was strongest in winter under the influence of the strong southeast winds that predominate in this season. During the summer, when northwesterly winds predominate, he found that the maximum surface temperatures occurred some distance from the coast, and suggested that this feature, together with the rising of σ_t-surfaces, was due to upwelling produced by offshore Ekman transport in the surface layers. Most of these features of the structure and currents have been confirmed in the more extensive series of observations now available (as will be shown later).

Of particular interest were the anomalous high temperatures attained by the surface and subsurface waters in 1957 and 1958. The influx of warmer waters along the west coast has been described by Tully et al. (1960). They were able to trace the movement of the water by the shift of isotherms on the 26.60 σ_t-surface which lies in the halocline below the level of the direct seasonal influence. The warmer waters could be detected to depths of 500 m along the continental slope and 250 m at Station P (Tabata, 1961).

OCEANOGRAPHIC CONDITIONS ALONG THE LINE

For the purpose of describing the oceanographic conditions along the Line, the seaward boundary of the coastal region as given by the 32.50‰ isopleth by Doe (1955) is not used in the present paper. Because of the annual cycle in surface salinity, the boundary undergoes considerable seasonal movement and is not convenient for the discussion of other properties. Instead, the Line is divided into two halves. The division is convenient because the structure and variations in the ocean as well as in the bottom topography (Fig. 3) differ

considerably from one half to the other. Moreover, the midpoint (135°W) is approximately the western limit of the coastal region as defined by Doe (1955) and Dodimead et al. (1963).

Properties of Water in the Upper Zone

The annual sequence of variation of surface temperature and the increase toward the coast throughout the year are shown in Fig. 9.

The annual cycle of temperature was nearly constant along the Line, the range being less than a degree greater in the western half than in the eastern half. The annual range at the various stations was about 6 C degrees in 1959, 7 C degrees in 1960, and 9 C degrees in 1961. Thus, the minimum and maximum temperatures occurred in 1961 and the lowest summer and highest winter temperatures in 1959. The winter minimum was attained in late March and the summer maximum in late August of each year. Where differences in phase could be detected, the extremes occurred slightly earlier in the eastern half of the Line. The temperature gradient along the Line remained relatively constant during both winter and summer. The average difference in temperature between Station P and Station 1 was 2.5 C degrees throughout the period of observation.

The coastward increase of temperature was not uniform but was usually interrupted by one or more pairs of relative maxima and minima. On several occasions, these features were strongly developed and persisted for several months. The more prominent maxima and minima are indicated in Fig. 9, and a typical record of the temperature along the Line, taken from the thermograph, is shown in Fig. 10. The relative extremes in temperature were observed most frequently in summer in the eastern half of the Line. They were found to occur anywhere along it.

It is believed that the occurrence of the relative maxima and minima is related to the presence of countercurrents or eddies in the general northward flow. However, the stations were spaced too far apart to make a critical comparison. Judging from the thermograph records, these features in the temperature structure had a horizontal scale of 50–100 km, whereas the station spacing was 150–300 km.

The uniformity of the annual variation of temperature along the Line indicates that the temperature is controlled primarily by the heat exchange across the surface and only secondarily by the winds and currents.

Near the continental shelf, the influence of seasonal changes in the wind system was apparent. Here, the annual range of temperature was less than to seaward. Maximum temperatures occurred about 300 km offshore. The lower temperatures near the coast are probably due to the upwelling of colder waters, as subsequent discussion will indicate. However, other factors could be important. Tidal movement and hence tidal mixing is more vigorous over the continental shelf, and fog is frequently formed along the coast in summer. Both may act to reduce the summer maximum temperature.

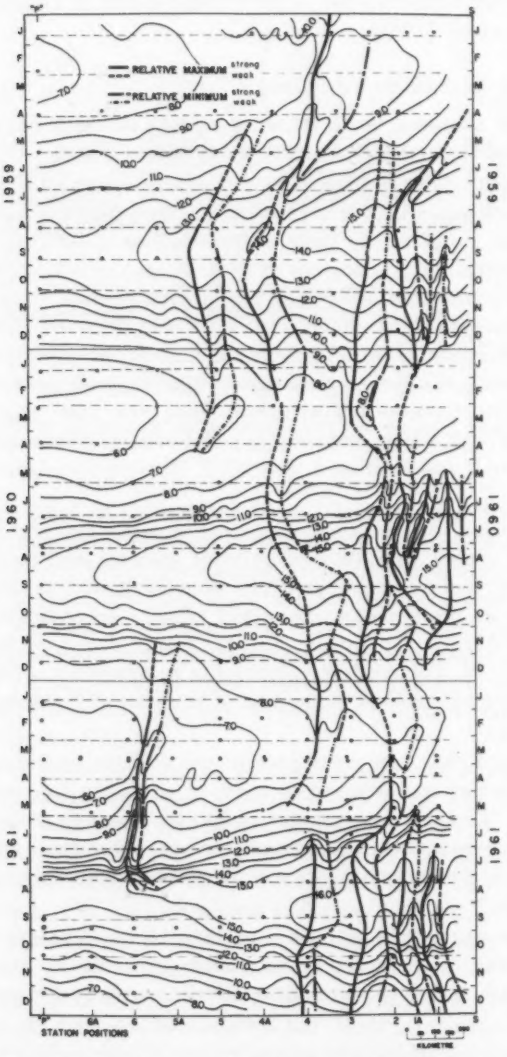


FIG. 9. Temperature (*C*) in the surface layer along the Line from January 1959 through December 1961. The temperatures were obtained from continuous thermograph records of ship's intake temperatures at a depth of about 3 m. The times of the weathership cruises are shown by the broken lines across the Figure. The locations and times at which stations were occupied are indicated by dots.

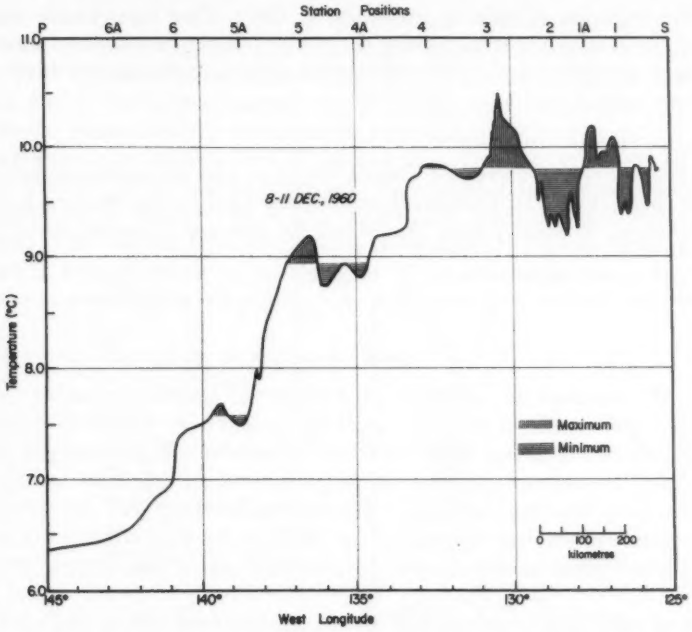


FIG. 10. An example of the relative minima and maxima observed in the thermograph records of intake temperatures along the Line. The pairs of relative maxima and minima were often strongly developed and appeared to persist for several months.

The decrease of salinity coastward along the Line was present throughout the period of observations (Fig. 11). Superimposed on this salinity gradient was an annual cycle of salinity variation in the coastward part of the Line that was related to drainage of fresh water from the land.

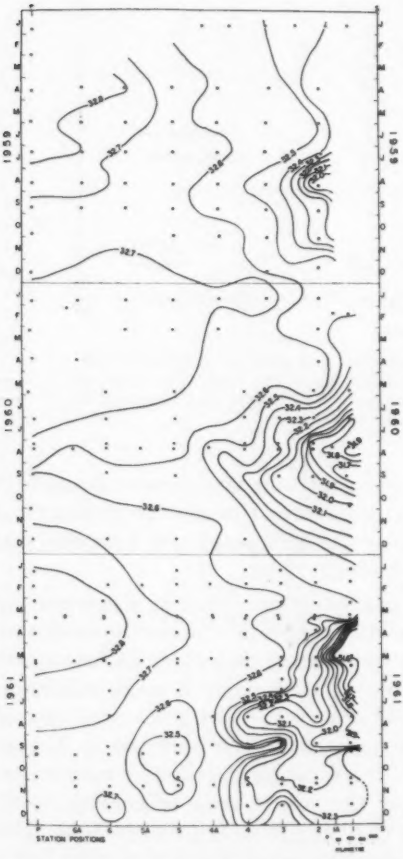
In the western half of the Line, the annual range of salinity was relatively small (0.2‰) and in phase with the temperature variation; the salinity minimum at the various stations coincided with the temperature maximum. In the eastern half, the salinity showed two minima during each year. A weak minimum occurred in spring (March–May) and was related to the winter maximum of rainfall in the coastal area. A more prominent minimum occurred in August and was related to river runoff from melting snow on the interior mountains. Herlinveaux and Tully (1961) state that the winter peak of coastal runoff occurs in January and the summer peak of river runoff occurred in June. The corresponding minima in salinity along the Line occur about 2 months later.

Because of the dilution of surface waters, the salinity gradient in the surface layer along the Line did not remain constant throughout the year. The gradient was about twice as large in summer as in winter. The appreciable dilution was confined to the eastern half of the Line.

The sequence of salinity variations in the surface layer in the coastward part of the Line was not as regular as that of temperature and appeared to be more directly influenced by the Ekman transports in the surface layer (as will be shown later).

DENSITY (σ_t)

The density (σ_t) decreased toward the coast and underwent an appreciable annual cycle (Fig. 12). Computations showed that in the western half of the Line, changes in density were dominated by changes in temperature. Salinity



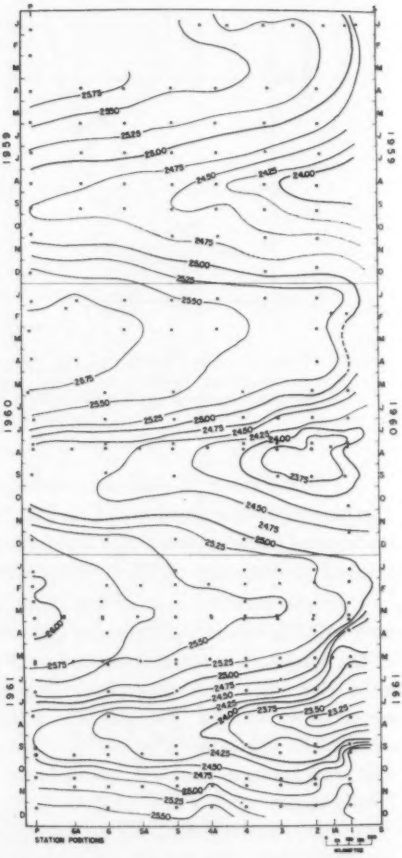


FIG. 11. *Left.* Salinity (‰) at 10 m depth along the Line during the study period. A prominent feature of the distribution was the annual cycle of dilution by coastal drainage in the eastern half of the line.

FIG. 12. *Right.* Density (σ_t) at 10 m depth along the Line during the study period. The variations are determined by the variations of temperature and salinity and are influenced by the processes affecting each of these variables.

variations contributed only 15% of the seasonal density variation. In the eastern half, particularly near the coast, the salinity changes accounted for about 40% of the density variations. The range of annual variation of density increased during the 3-year period of observation and reflected the increase in the annual ranges of both temperature and salinity.

During summer, the gradient of density along the Line increased to about twice its winter value. This increase was due entirely to the increase in the salinity gradient in summer. As will be shown, the effect of the annual change of gradient in the surface layer on the pressure gradient in the underlying water was offset to a large extent by the rising of σ_t surfaces below the surface layer in summer and sinking in winter.

Properties of Water in the Lower Zone

The increase of temperature toward the coast for the lower zone is evident in the vertical section of temperature along the Line for May 1961 (Fig. 13).

The temperature was relatively constant in the halocline and occasionally an isothermal layer or an inversion, which was not continuous, was found within this layer. The temperature below the halocline decreased gradually with depth to a minimum of 1.5 C at 4000 m. Isothermal surfaces generally sloped downward toward the coast, dropping by about 400 m from Station P to Station 1.

A halocline in the depth range of 100–200 m was a prominent feature of the salinity distribution along the Line (Fig. 14).

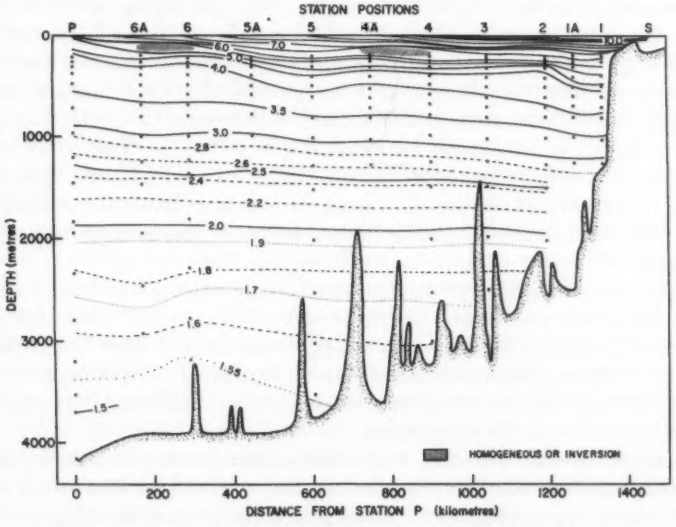


FIG. 13. An example of a vertical section of temperature (C) along the Line. This section is based on a cruise carried out in May 1961 by the C.N.A.V. *Oshawa*.

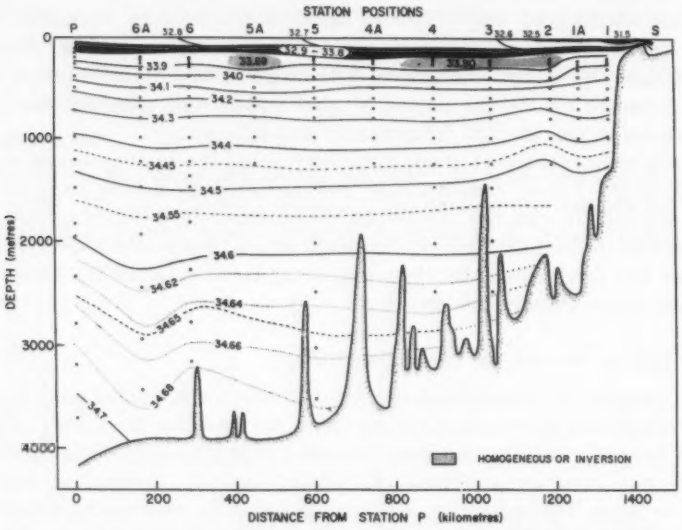


FIG. 14. An example of a vertical section of salinity (‰) along the Line. This section is based on a cruise carried out in May 1961 by the C.N.A.V. *Oshawa*.

The decrease of salinity toward the coast was noticeable only in the western half of the Line. In the eastern half, the surfaces of constant salinity had a generally upward slope toward the coast, providing a net increase of salinity from Station P to Station 1. The coastward rise of the surfaces of constant salinity was often masked by alternate regions of sharper rise and fall.

A layer of nearly constant salinity, occasionally with a slight inversion (<0.015‰; salinity decreasing with depth) was frequently found immediately below the halocline at depths between 200 and 300 m. This layer was not continuous, and was found to occur anywhere along the Line. During 1959 and 1960 it occurred at Station P. The layer was stabilized by a corresponding decrease of temperature with depth. However, the stability reached a minimum in this layer.

The density in the lower zone generally decreased toward the coast (Fig. 15). The decrease was largest in the western half of the Line and showed considerable variation. However, at times, the horizontal density gradient was the reverse of those observed generally, as during May 1961 at depths between 1200 and 2000 m. Such reversals were temporary but indicated that appreciable variation can occur in the deep water.

The slight upward slope of the isohaline surfaces toward the coast (Fig. 14) was not sufficient to offset the effect of the much larger downward slope of the isothermal surfaces (Fig. 13) in determining the density distribution. Hence, surfaces of constant σ_t sloped downward toward the coast, dropping by nearly 200 m from Station P to Station 1.

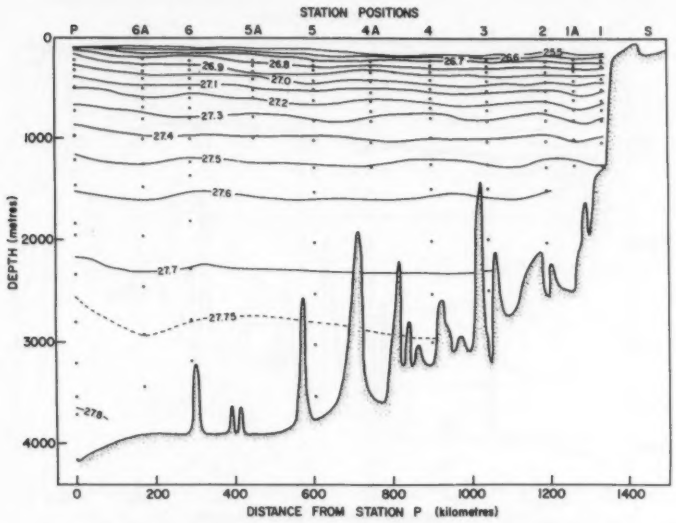


FIG. 15. An example of a vertical section of density (σ_t) along the Line. It is based on cruise data obtained in May 1961 by the C.N.A.V. *Oshawa*.

The dissolved oxygen content decreased from near-saturation values at the surface (about 0.6 mg-at/litre) to a minimum of 0.03–0.06 mg-at/litre at depths of 800–1200 m (Fig. 16). Below the minimum, the dissolved oxygen content increased gradually with depth reaching about one-half the upper zone value at the bottom. The minimum values decreased and the depth of the minimum surface generally rose toward the coast. The changes with time were not examined closely because of the lack of observations of dissolved oxygen content along the Line. The temporal variations of salinity, temperature, and dissolved oxygen content of the water in the upper 1000 m at Station P from the summer of 1956 through that of 1962 have been described by Tabata (MS, 1964).

The deep waters of the lower zone along the Line exhibited relatively small variations of properties when compared with the upper zone. The changes that occurred with time were not much larger than errors inherent in the determination of the properties and these changes cannot be determined with complete confidence. Nevertheless, slopes of surfaces of constant temperature, salinity, and density are discernible even at 3500-m depth in the western half of the Line (Fig. 13, 14, and 15). The marked change of salinity from Station P to Station 6A is probably real, as similar changes in deep-water salinities were observed during the same cruise between a pair of stations to the north of Station P and again a few hundred kilometres to the west of the Station. However, all stations close to Station P position yielded lower values for the deep-water salinities. The higher salinities at Station P (if real) must be due to a relatively narrow "tongue" of high salinity water extending from the north.

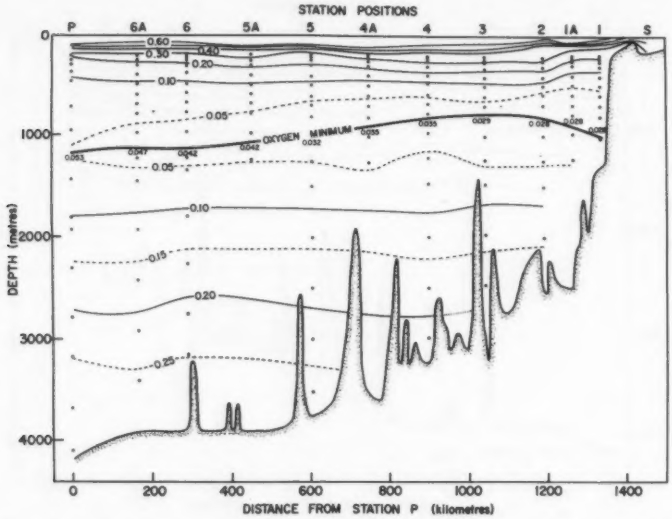


FIG. 16. An example of a vertical section of the dissolved oxygen content (mg-at/litre) along the Line. This section is based on a cruise carried out by the C.N.A.V. *Oshawa* in May 1961.

Annual cycles in the variations of temperature and salinity along an approximately level surface (200 m; Fig. 17, 18) were clearly discernible in the eastern half of the Line, though the 200-m surface is well below the level of direct seasonal influence. The annual cycles appeared to be produced chiefly by the vertical migration of the 26.80 σ_t surfaces (Fig. 19) — rising in summer and sinking in winter. Though the annual cycle of temperature was not recognizable at the 26.80 isopycnal surface (Fig. 20), the annual cycle of depth of the σ_t surface was evident.

In the western half of the Line, the annual cycle was small and not readily discernible against the background of both shorter and longer period variations. To illustrate the longer period changes further, the variations of temperature, salinity, and dissolved oxygen content at standard depths at Station P are shown, respectively, in Fig. 21, 22, and 23. These figures show that large systematic changes of all three variables occur in the halocline and lower zone waters. High temperatures occurred during the "warm years" of 1957 and 1958 (see for example, Tully et al., 1960), but these were exceeded at all depths below the upper zone in 1960. Dissolved oxygen content, however, was high in 1957–58 but low in 1960. The changes of salinity were less marked and the extremes did not coincide in time with those of temperature and dissolved oxygen content. These nonseasonal changes in the deeper water were not likely to be produced by local processes and were attributed to the advection of water having different characteristics than at Station P. A detailed examination of the effect of advection at Station P bears this out (Tabata, MS, 1964).

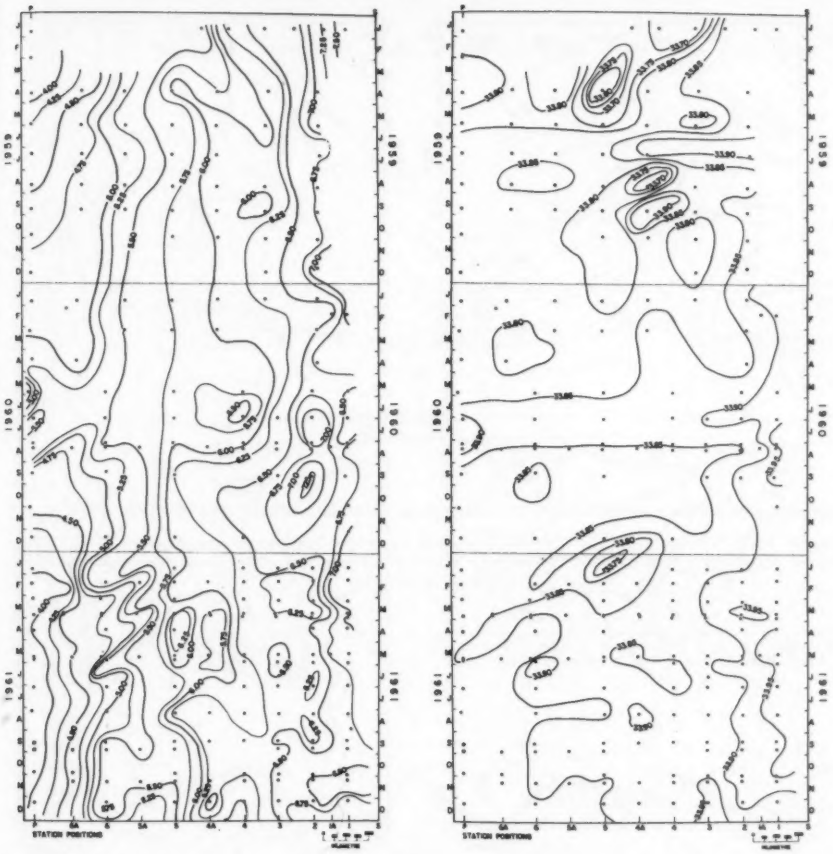
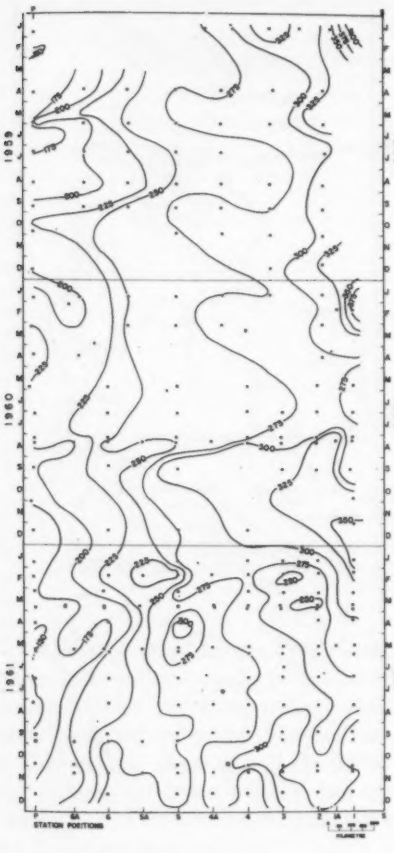


FIG. 17. *Left.* Distribution of temperature (C) on the 200-m surface along the Line during the study period.

FIG. 18. *Right.* Distribution of salinity (‰) on the 200-m surface along the Line during the study period.

WIND-INDUCED TRANSPORTS

The Line crosses the northern branch of the flow originating from the Subarctic Current and the West Wind Drift (Fig. 8). The branch continues northward around the Gulf of Alaska to feed the intensified westward current (Alaskan Stream) along the Aleutian Islands. This flow is part of the system of eastern boundary currents along the west coast of North America and is of considerable theoretical interest. According to the steady-state theories of ocean circulation developed by Sverdrup (1947) and Munk (1950), the eastern boundary current does not become intensified in the manner of the western boundary currents but is in local equilibrium with the mean wind stress acting on the



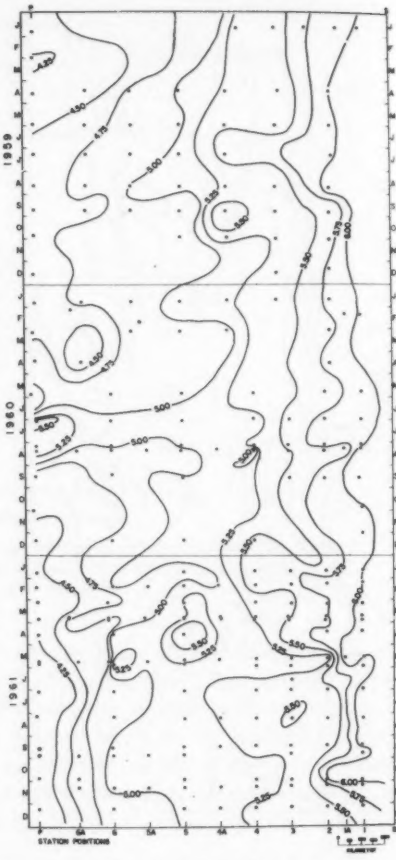


FIG. 19. *Left.* Depth (m) of the 26.80 σ_t surface along the Line during the study period.

FIG. 20. *Right.* Distribution of temperature (C) on the 26.80 σ_t surface along the Line during the study period.

surface. In a recent theoretical study by Carrier and Robinson (1962), it was suggested that the flow into the Gulf of Alaska might be partially inertial in character. The suggestion was not substantiated by the present series of observations (as will be shown subsequently). The Subarctic Current and the West Wind Drift are located in the vicinity of maximum zonal wind stress (zero curl) rather than maximum curl of the stress. The northern branch flowing into the Gulf of Alaska cannot be included in the Carrier and Robinson model. The features of the wind system and of the flow in the region of the Line appear to be more consistent with the assumption of local equilibrium. Their model is also in direct conflict with the conclusion reached by Stewart (1964) that no boundary transport can exist on the eastern side of an ocean unless it

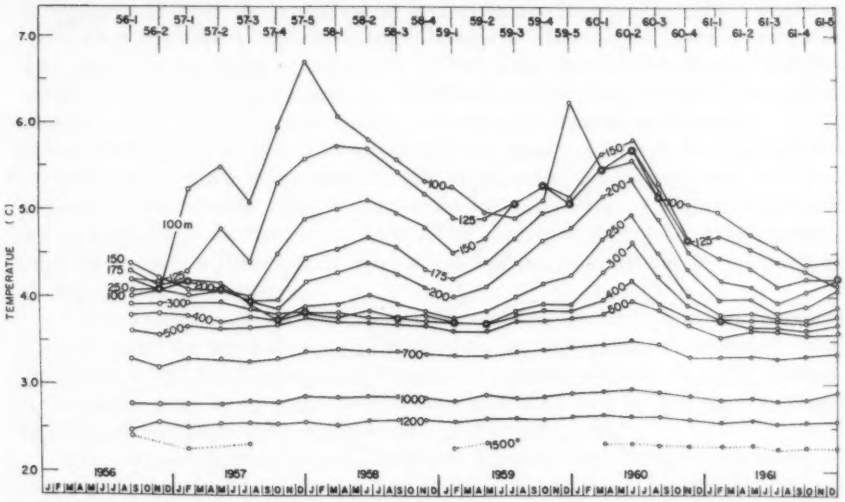


FIG. 21. Temperatures at selected standard depths (100–1500 m) at Station P from August 1956 through December 1961. The points represent averages of observations during the 6-week periods on station.

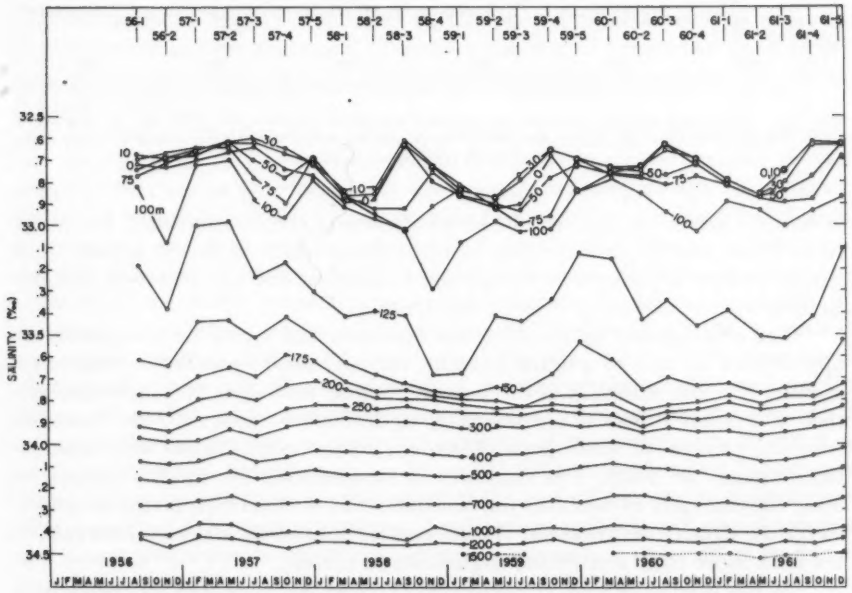


FIG. 22. Salinities at selected standard depths (100–1500 m) at Station P from August 1956 through December 1961. The points represent averages of observations during the 6-week periods on station.

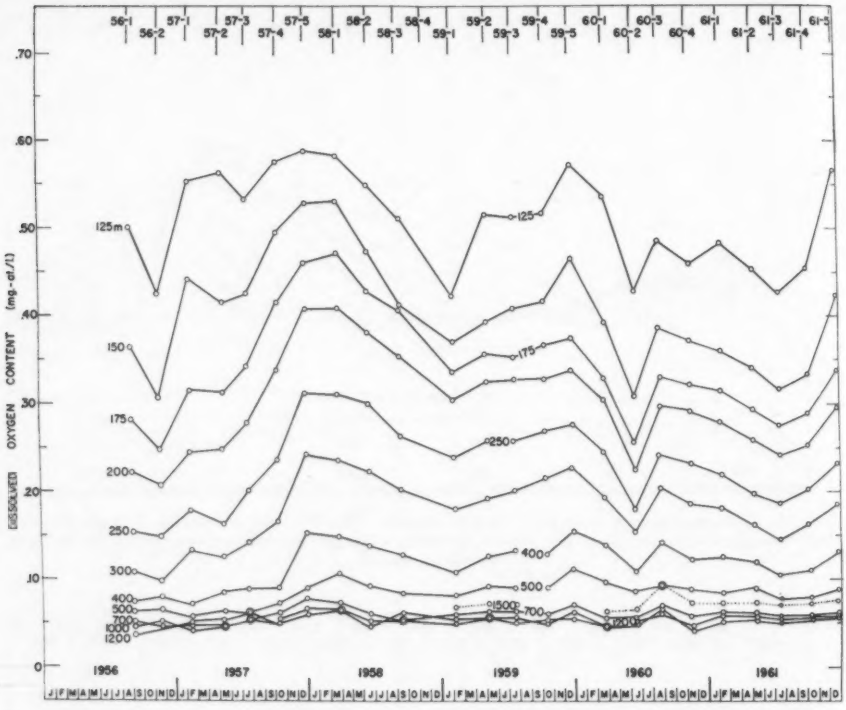


FIG. 23. Dissolved oxygen contents at selected standard depths (125–1500 m) at Station P from August 1956 through December 1961. The points represent averages of observations during the 6-week periods on station.

is directly driven by local winds. However, one of the more striking characteristics of the flow is its variability in time. The variations do not appear to be simply related to changes in the applied wind stress and are, therefore, difficult to understand in terms of present-day theories.

The observations taken along the Line over the 3-year period provide an opportunity to test, to a limited extent, the conclusions reached in theoretical studies. A major difficulty is the present rudimentary state of time-dependent theory. Present theory is not adequate to derive the expected flow variations from the changes of wind stress. Thus, the critical comparisons with observations cannot be made. The difficulty is compounded by the inadequacy of observational data to establish the flow field and its variations, except as reflected by the density distribution. Hence, comparisons of theory and observations are little more than plausibility arguments at present.

As has been shown by Fofonoff (1962) the influence of winds blowing over the surface of the ocean can be interpreted in terms of a variable tangential wind stress acting on the ocean surface. The surface stress induces motion of

the water both near the surface and in the deeper layers. The direct effect of the surface stress is to induce a flow of mass in the surface layer directed at right angles to the right of the stress vector. This flow is termed the "Ekman transport" and is proportional to the magnitude of the stress vector. A secondary influence on the deeper layers arises from the convergence or divergence of the Ekman transport. If the Ekman transport is not uniform horizontally, a vertical motion must be present to compensate for the divergences and convergences. The vertical flow is downward for a convergent Ekman transport and upward for a divergent transport. The vertical velocity is nearly proportional to the curl of the surface wind stress and is upward if the curl is positive (divergent Ekman transport).

The response time, for the Ekman transport in the surface layers to adjust to changes in the wind stress, is believed to be very short — probably comparable to a half-pendulum day. In terms of seasonal or even monthly changes in the mean wind stress, both the Ekman transport and its divergence can be regarded as responding instantaneously to the variations.

The vertical motion, into or out of the Ekman layer, changes the potential vorticity of the underlying water and induces a meridional component of flow necessary to offset the changes in potential vorticity. According to Veronis and Stommel (1956), the deeper layers respond barotropically, except for extremely slow changes. Thus, for changes of the order of an annual period and shorter, the meridional component of flow is imparted to the water at all depths, regardless of the density structure. In the present discussion, it is assumed that monthly and even seasonal changes in the curl of the surface wind stress produce instantaneous changes in the barotropic flow throughout the entire column of water.

Generally, the long-term mean of the curl of the surface wind stress is not zero and the mean flow into or out of the Ekman layer determines the mean circulation in any locality in the ocean, according to the steady-state theory of Sverdrup (1947). Therefore, it is assumed that the mean circulation represents a balanced state in which the mean vertical flow is balanced by vertical diffusion of heat and salt, and by horizontal advection, so that the surfaces of constant properties are not displaced. However, variations of seasonal and shorter periods are assumed to be capable of producing vertical migrations of the surfaces of constant properties. Hence, time-dependent changes should be related to deviations of the curl of the wind stress from its mean. Moreover, such changes should be cumulative within the baroclinic response time of the ocean. Hence, an estimate of the variation of depth of a surface of constant property, e.g. an isopycnal surface, can be made by integrating, with respect to time, the difference of the vertical velocity from its mean. The averaging time chosen to determine the mean velocity will also determine the lowest frequency at which the barotropic response is expected to be dominant. It is assumed to be of the order of a year or longer.

Changes of the depth of isopycnal surfaces will be reflected in the variations of the dynamic height anomaly. If the vertical velocity (upward), as estimated

from the curl of the wind stress, is less than its mean, the isopycnal surfaces will move downward and the dynamic height will increase. Conversely, if the velocity is greater than its mean, the surfaces will move upward and the dynamic height will decrease. Then, because the anomaly of dynamic height is an integrated measure of the position of isopycnal surfaces, it can be used for comparison with the calculated vertical displacements of the density field.

A complicating factor is the horizontal shift of the density field by the barotropic components of motion. If the constant-property surfaces are not horizontal, advection by the barotropic motion can offset or enhance the local vertical displacement. A further complication can arise from instabilities of the flow. If dynamical instabilities can occur, the variations of the flow need not be correlated with variations of the stress field.

In the presence of a coastal barrier, the divergence of the Ekman transport is dominated by the component of transport normal to the coast rather than by the curl of the wind stress. The curl tends to reach a maximum during the period of strong winter winds, whereas the offshore component of transport reaches a maximum in summer. Thus, an appreciable difference in the response of the ocean can be anticipated near coastal boundaries as compared with the offshore regions.

Wind Systems in the Northeast Pacific Ocean

Before attempting to discuss wind-induced transports along the line in detail, it will be useful to describe the general wind system in the Northeast Pacific Ocean, as this would have a bearing on the subject.

The mean surface winds in the Gulf of Alaska reflect the annual cycle of development of the Aleutian Low and the North Pacific High atmospheric pressure regions of the North Pacific (Fig. 24). The cycle has been described in terms of the January and July mean sea-level pressures by Haurwitz and Austin (1944). In winter, the Aleutian Low is strongly developed in the Gulf of Alaska and the mean air flow is directed northward, almost parallel to the coast. As summer approaches, the Low weakens and shifts westward. The North Pacific High expands westward and northward until, in summer, it dominates the entire eastern half of the North Pacific. The accompanying air flow shifts direction so that during the summer months the winds are from the west or northwest in the Gulf of Alaska. The summer winds decrease to about half the wind speed attained during the winter months.

Along the Line, the dominant annual cycle in the mean winds is produced by the Aleutian Low "pressing" against the coastal mountains in winter, giving rise to a strong increase in the meridional component of the wind. The zonal component shows little change. The increased zonal gradient of the mean atmospheric pressure, which gives the appearance of the Low being compressed against the coast, is presumably due to the tendency for winter cyclones to be deflected by the coastal mountains so that their paths tend to lie parallel to the mountain range or to curve sharply near the coast. Haurwitz and

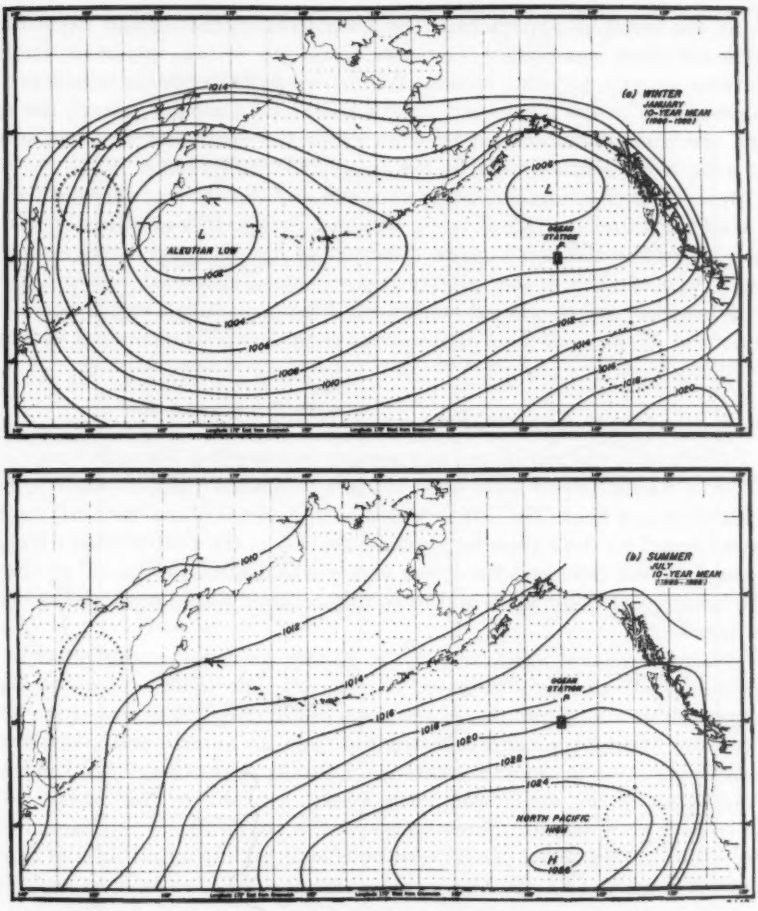


FIG. 24. Distribution of atmospheric pressure (millibars) at sea level over North Pacific Ocean in winter and summer.

Austin (1944) attribute the failure of the Low to penetrate inland, at least in part, to the coastal mountains.

At Station P, the annual cycle in speed and direction is less than in the vicinity of the coast. Winds are predominantly from the west but with a higher percentage of south-west winds during the summer. The mean wind speed in winter (about 13 m/sec) is twice the summer mean. The weakest mean winds occur from May through September and the strongest, from October through February.[4]

[4]Climatology Division, Meteorological Branch, Department of Transport of Canada. *Ocean Station "P."* Detail listing of marine observations with daily sums of selected parameters, and mean daily, weekly, fortnightly, and monthly values — January 1955 — December 1961. Unpublished manuscripts.

As the coast is approached, the amplitude of the annual cycle of the meridional wind component increases, resulting in an increased range of directions as well as wind speeds. Winter winds shift to the southeast and summer winds to the northwest. At the coast of Vancouver Island, the mean winds are from the southeast in winter and the northwest in summer with only weak components normal to the coast (Doe, 1955; Lane, 1962).

Because of the annual changes of speed and direction of the mean winds, the wind variations contain a strong semiannual as well as an annual variation. The wind passes through a given direction within its range twice each year, yielding two maxima in the velocity components. The semiannual variation is not as regular as the annual variation. The semiannual harmonic is more pronounced in the variations of the stress components as compared with wind velocities.

Ekman Transport Components

Estimates of the meridional and zonal components of Ekman transport for the North Pacific Ocean have been made by Fofonoff (MS, 1960; MS, 1961) and Fofonoff and Ross (MS, 1962) from monthly means of sea-level atmospheric pressure issued by the Extended Forecast Section of the United States Weather Bureau. In these estimates the stress vector was assumed to lie 15° to the left of the mean geostrophic wind and to be proportional to the square of the mean wind speed.

The mean annual variations of the Ekman transport components in the vicinity of the Line are illustrated in Fig. 25. The points represent 7-year averages (1955–61) by months, of the zonal and meridional components. These points were fitted by a curve constructed from an annual and a semiannual sinusoidal variation to show the relative amplitudes of the two cycles. Evidently, the amplitude of the semiannual cycle was comparable to that of the annual cycle in both components. The increase of the amplitude of the annual cycle as the coast is approached can be seen by comparing the amplitudes at Station P (145° W) and at 130° W. The increase, about 400%, was almost entirely in the annual cycle. The semiannual cycle and the mean showed relatively little change. The meridional component, produced by the zonal component of stress, decreased slightly near the coast.

Considerable variations in the directions and magnitudes of the Ekman transports occurred from month to month and from year to year (Fig. 26). Along the coast they are usually directed offshore during the heating season (April through September) Strong onshore transports occurred during the remaining season.

The response of the coastal water to the Ekman transport is obvious. An offshore Ekman transport would move the water offshore. This creates a divergence area that necessitates a demand for water in the surface layers. From continuity considerations, this demand may be satisfied by advection of water into the area or by the rising of deep waters, or both. When onshore Ekman transport is effective, the surface waters would accumulate along the

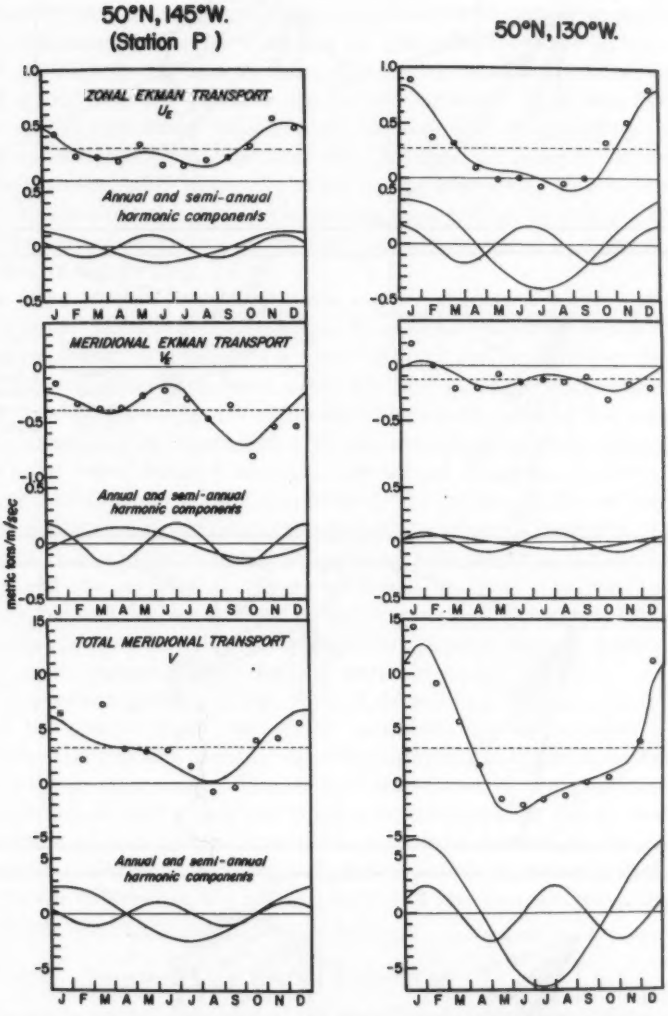


FIG. 25. The annual cycles of zonal and meridional Ekman transport components and the total meridional transport at 50°N, 130°W, and at Station P. The transports were estimated from monthly means of sea-level atmospheric pressure. A strong annual and semiannual cyclic variation in the transport components was expected and the amplitude and phase of the variations were estimated by harmonic analysis of the components over a 7-year period (1955–61).

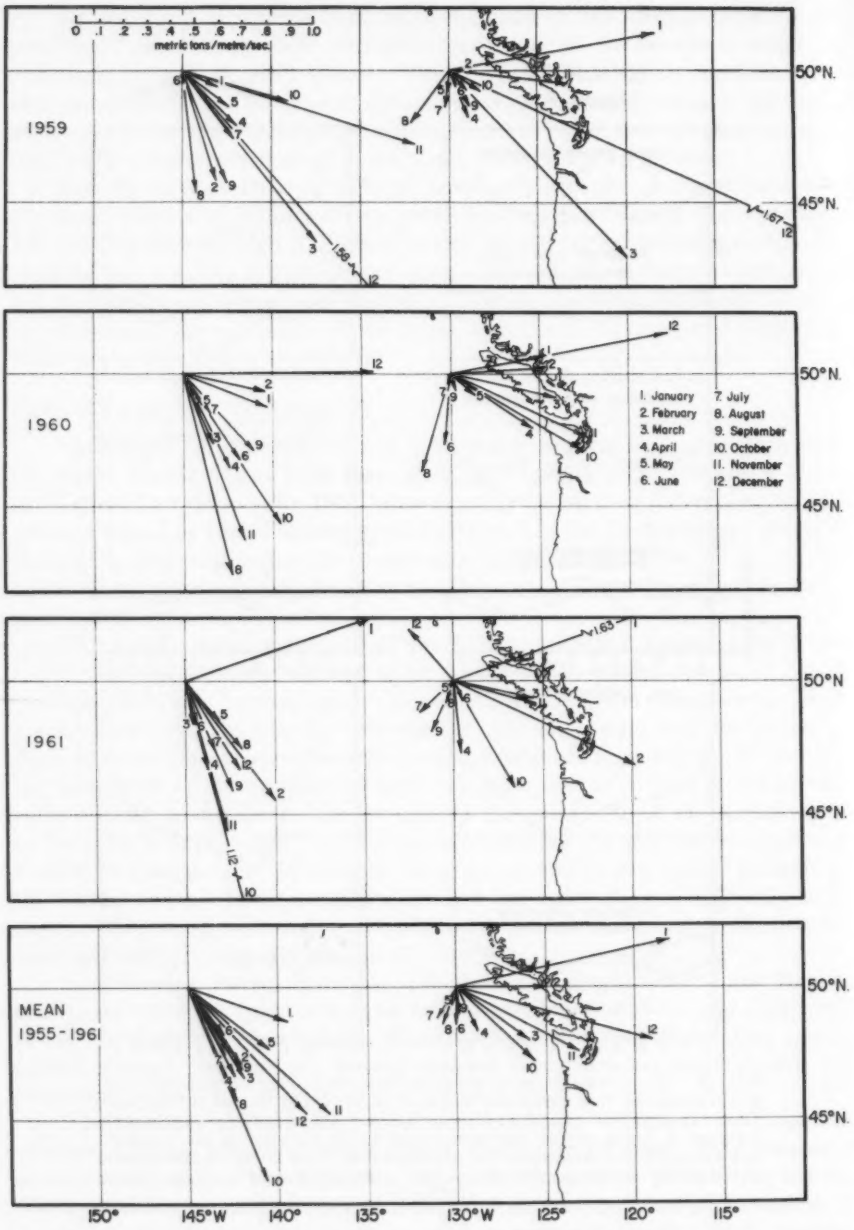


FIG. 26. Monthly Ekman transport vectors at 50°N, 130°W, and Station P for the study period and for a 7-year mean.

coast, thus creating a convergence area. The accumulated water may result in thickening the surface layers and displacing the deeper water offshore, or permit the removal of this accumulated water by advection of surface waters, or both.

The influence of Ekman transport on the surface waters off the coast is revealed in Fig. 9. As mentioned earlier, maximum surface temperatures occurred about 300 km offshore during the summer. This was due to the presence of the cold water adjacent to the coast due to upwelling under the influence of offshore Ekman transport (Fig. 26) brought about by the northwest winds that predominate along the coast during this time. Due to the annual cycle of the Ekman transport in the coastal region (Fig. 25 and 26) an annual cycle of the vertical migration of isopycnal surface occurred — rising in summer and sinking in winter (Fig. 19).

The effect of this transport on the surface salinity was more readily apparent than in the surface temperature. As evident from comparison of Fig. 11 and 26, strong offshore transports occurred in April and from July to September. Diluted surface water was more extensive and persisted longer than in the previous 2 years. Similarly, the retreat of the diluted water in late autumn and winter appeared to be connected with the magnitude of the onshore Ekman transport. In 1959, onshore transport developed strongly in November and December coinciding with a sharp increase in the salinity of the surface layer. In 1960, the onshore transport remained relatively weak until December. Unfortunately, salinity observations are lacking during this period in the eastern half of the Line and the correlation cannot be made. In 1961, transport toward the coast occurred only in November. During October and December, the transport was parallel to the shore. As a consequence, diluted surface water persisted over the entire eastern half of the Line until the end of the year.

A by-product of the vertical shift of density surfaces was the reversal of phase of the temperature and salinity variations below the upper zone. The lowest temperatures and greatest salinities occurred in summer, rather than in winter as in the upper zone. The vertical movement of the isopycnal surfaces in the subsurface layers near the coast was attributed to the annual cycle in the variation of the Ekman transport component normal to the coast. The transport was offshore in summer and onshore in winter, causing depletion and accumulation of water in the surface layer and the compensating rise and fall of the isopycnals in the underlying layers.

Meridional Component of Total Transport

The mean annual variation of the meridional component of total transport is shown in Fig. 25. This component is directly proportional to the curl of the surface wind stress. The total transport component underwent a strong annual variation and change of sign. As in the zonal Ekman component, the amplitude of the annual cycle increased toward the coast. The mean (shown by dashed line in Fig. 25) and the semiannual cycle showed smaller changes. The semiannual cycle was slightly less prominent in the total transports than in the Ekman transports.

The vertical flow into the Ekman layer is proportional to the difference between the total meridional transport and the meridional Ekman transport. As the total meridional transport was usually much larger than the Ekman component, the vertical velocity could be estimated with sufficient precision from the total meridional component. Thus, a northward flow of 10 metric tons per metre per second corresponded to an upward flow into the Ekman layer of 3.4 m/month or about 41 m/year at 50°N. During the summer, the mean curl of the wind stress was negative. The vertical motion was downward, out of the Ekman layer, and the total meridional transport was southward. In winter, the vertical motion was upward and the total meridional transport northward. The annual mean transport was northward.

DYNAMICS

A discussion of the dynamics of currents and transports of any ocean region based only on hydrographic station data (density observations) is necessarily limited because of the absence of observations of the barotropic mode of motion. Much of the time-dependent response of the ocean probably occurs in the barotropic mode. Hence, a discussion of the variations of the baroclinic flow, as represented by observed changes in the density field, can be made only by assuming interactions between the barotropic and baroclinic modes.

The baroclinic, or relative geostrophic flow is related to the density field in the ocean and can be examined in terms of the anomalies of dynamic height and/or potential energy (Fofonoff, 1962). Changes in the magnitude of these quantities at a given position in the ocean can be determined from local changes of density and reflect the vertical movement of isopycnal surfaces caused by the local divergence of the Ekman transport, by horizontal advection, and by mixing and diffusion. Estimates of local Ekman divergence and the resultant vertical velocity can be made readily from transport values computed for the North Pacific Ocean (Fofonoff, MS, 1960, MS, 1961; Fofonoff and Ross, MS, 1962). These estimates were made from the mean atmospheric pressure over the ocean and involve assumptions about the relation of mean surface winds to the pressure gradients in the atmosphere and to the mean wind stress on the ocean surface (Fofonoff, 1962).

The accuracy of the estimates is open to question. Probably the most serious shortcoming is the use of monthly means of sea-level pressure. The estimated mean transports and divergence are not reliable if the wind changes direction and magnitude frequently within the monthly period. However, the estimates could be computed from averages over a shorter period, for example, from 5-day means or even from daily pressure charts. Hence, the possibility of improving the estimates of surface stress and of the resultant induced transports makes it necessary to examine the applicability of these estimates in interpreting the measured changes of structure and circulation in the ocean. At the present time, neither the stress estimates nor the measurements of

flow in the ocean are sufficiently comprehensive or reliable to permit a critical evaluation of the method. A more detailed discussion of the assumptions and procedures used in estimating the mean surface stress and the baroclinic transports was given by Fofonoff (1962).

Dynamic Height Anomaly

The anomaly of dynamic height in the Northeast Pacific is minimum at the centre of the Alaska Gyre, to the northwest of Station P (Fig. 7). From this minimum, the anomaly increases to the south and toward the coast, indicating the general cyclonic circulation around the Gulf of Alaska. Along the Line, the dynamic height anomaly increases toward the coast, reaching a maximum near the continental shelf.

The distribution of the anomaly of dynamic height, at the surface relative to the 1000-m surface, along the Line during the study period is shown in Fig. 27. In this figure, lines of constant dynamic height anomaly are *not* streamlines in the time-distance plane. The gradient of the anomaly with respect to horizontal distance at a specific time is proportional to the geostrophic current at the surface relative to the current at 1000 m. However, the gradient with respect to time has no simple dynamic interpretation. Countercurrents along the Line are indicated by closed or reentrant curves that cross the Line more than once at a specific time. The occurrence and structure of the northward current vectors and southward countercurrent vectors across the Line are shown in Fig. 28, which can be used as an aid to the interpretation of Fig. 27.

The anomaly showed a recognizable annual variation in the eastern half of the Line. The anomaly reached a minimum in late spring or early summer and a maximum in later summer or during the autumn. The maximum anomaly occurred near shore at Station 2 or 3. Nearer the coast (Station 1), the anomaly was influenced by the Ekman transport normal to the coast. In winter, when onshore transport occurred, the anomaly at Station 1 was greater than at Station 2 due to accumulation of lighter water at the coast. This phenomenon was reflected in the sinking of density (σ_t) surfaces (Fig. 19). In summer, Station 1 usually had a lesser anomaly than Station 2 as lighter water was carried seaward by the offshore transport. Thus, the relative geostrophic current along the continental slope tended to be northward in winter and southward in summer in correspondence with the changes of wind direction. However, the sequence was not regular as can be seen from Fig. 27 and 28.

Further offshore, the variation of dynamic height appeared to correspond to some extent with the cumulative vertical displacement calculated from the local divergence of the Ekman transport. It was assumed earlier that the vertical displacement of surfaces of constant properties would be produced by departures of the vertical velocity from its mean. Thus, by integrating the difference of vertical velocity from its mean with respect to time, the change of position of the surface can be estimated. Such a calculation has been carried out and is presented in Fig. 29, together with the observed dynamic height

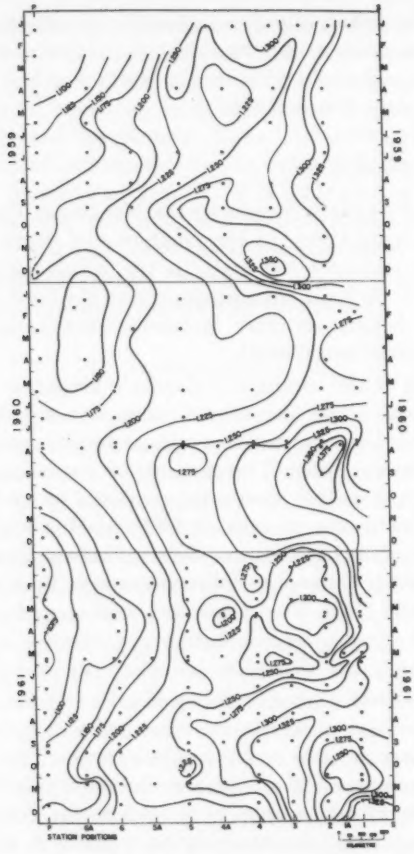


FIG. 27. Dynamic height anomaly at the surface relative to the 1000-m surface (0/1000 decibars) along the Line during the study period. The differences in the anomaly at a given time are proportional to the geostrophic current at the surface relative to that on the 1000-m surface. However, differences in time have no simple dynamical interpretation.

anomaly at several stations along the Line. The total ranges of the calculated vertical displacements were of the order of 10 m, assuming no net displacement over the 3-year period. Such small vertical movements would produce changes in the dynamic height that would scarcely exceed the short-term variability and errors of measurement, and could not be considered significant. The observed vertical movements of the isopycnal surfaces (Fig. 19) showed considerable similarity of shape to the calculated vertical displacement curve but were about 10 times the calculated values (nearly 100 m in the upper 1000 m of the

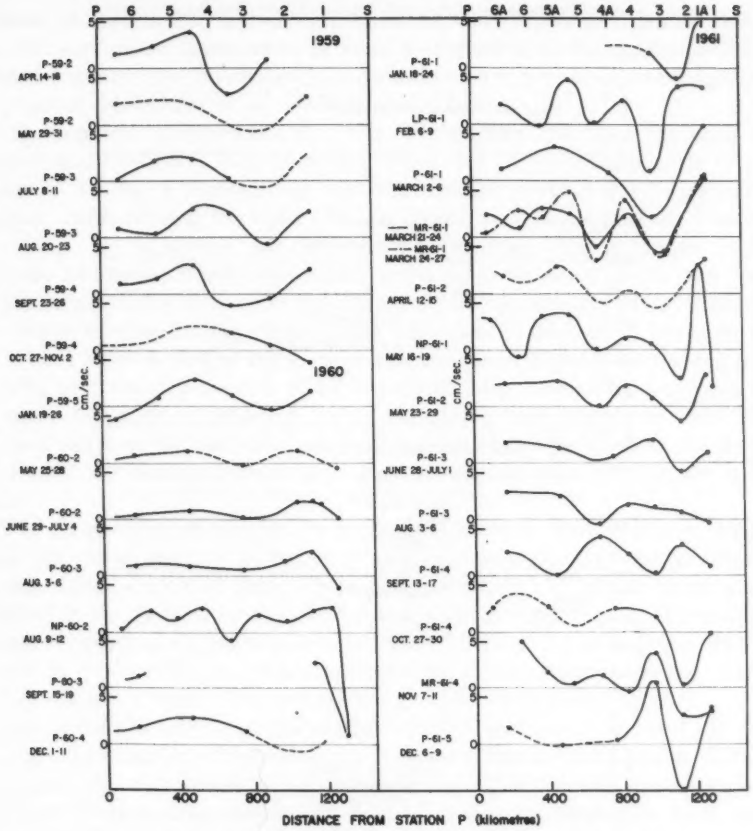


FIG. 28. Vectors of geostrophic current normal to the Line at the surface relative to the 1000-m surface (0/1000 decibars). The surface currents showed a complex structure of countercurrents that changed with time. Countercurrents occurred most frequently in the eastern half of the Line.

ocean). (In another study, Tabata (MS, 1964) found that by assuming no net displacement over a 7-year period (1955–61), the computed displacement at Station P was about twice that estimated from the 3-year assumption. During the years from summer 1956 through summer 1962, the observed vertical movement of isopycnal surfaces was correlated with the calculated displacement except during the 1-year period between the summers of 1958 and 1959.)

A convincing explanation of the discrepancy was not found. It does not appear reasonable to assume that the wind stress is underestimated by an order of magnitude. If the amplified response of the ocean is due to horizontal shifts of the density field, the upward displacements should correspond to a southeast movement of water (because of the presence of the "dome" to the

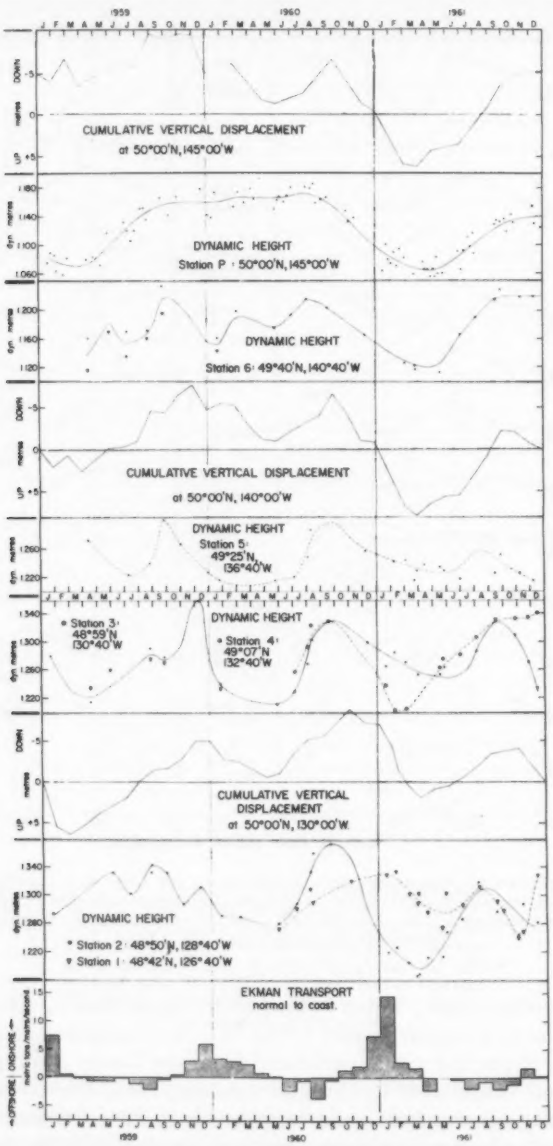


FIG. 29. A comparison of the cumulative vertical displacement of surfaces of constant properties, calculated by integrating the deviation of the vertical velocity from its mean, and the observed variations of the anomaly of dynamic height along the Line. (The observed variations of dynamic height can be interpreted in terms of the vertical displacement of surfaces of constant density. The observed vertical displacements show considerable similarity to the calculated displacements, but are about 10 times as large as calculated.)

northwest of Station P). Such a shift, however, would have to occur in winter, when the northward component of total transport is at its maximum. The amplified response may be related in some unknown way to bottom topography or to possible resonances or instabilities of the flow.

The dynamic height anomaly (Fig. 27) showed the least variation at Station 1 and Station 5. The decreased amplitude of the annual cycle of the anomaly at Station 1 is consistent with the changes of the normal component of Ekman transport near the coast (Fig. 25), which tend to increase the dynamic height (Fig. 27) in winter and decrease it in summer. The reduction in the amplitude at Station 5 indicates that a nodal point for some of the baroclinic changes occurs in the vicinity of the Station. This corresponds to the tendency for a phase reversal in the temperature variation at Station 5, between the western and eastern part of the Line as can be seen in Fig. 17. Increasing temperatures at Station P tended to be associated with decreasing temperatures near the coast, such as occurred during the spring and summer of 1960. The decreased range of the variations at Station 5 could be due to some effect connected with the bottom topography (Fig. 3) but the specific influence is not known.

The geostrophic vectors at the surface, relative to 1000 decibars, defined in Fig. 28 show systems of currents and counter currents that persist for relatively long periods of time. These may correspond to eddies or meanders imbedded in the general northward flow such as shown in Fig. 7. The countercurrents occurred most frequently to the east of Station 3 and between Stations 4A and 5. Frequently, as many as three separate countercurrents, spaced 300–500 km apart, were indicated by the gradient of dynamic height.

The wave-like profile of the surface current vectors along the Line (Fig. 28) raises the question of the existence of Rossby waves in the region. It could be assumed that the waves are barotropic and that the particle motions within the waves are distorting the density field by horizontal advection. Such interactions in this so-called Transitional region where a meridional temperature gradient occurs might yield observable baroclinic effects such as the development of the countercurrents in the geostrophic flow. It was calculated from the theory (Veronis and Stommel, 1956) that, if the wavelength is comparable to the distance between the observed countercurrents (300–500 km), the phase velocity of the waves would be about 4–10 cm/sec and the period about 2–3 months. Because of the decrease of depth toward the coast, the waves would tend to move northward rather than westward in the vicinity of the coast. It was concluded that, as the mean flow across the Line is also northward, it is not likely that stationary Rossby waves could exist in the region.

The question of the presence of Rossby waves in the region of the Line cannot be answered in terms of the observations currently available. Theoretical understanding of the behaviour of the waves in the presence of coastlines and variable bottom topography is not adequate to depict the critical characteristics necessary for identification. Observations were not adequate to determine whether the countercurrents along the Line are closed eddies or a meandering

flow. The frequency of observations in time was insufficient to show the development and movement of the system of currents.

Potential Energy Anomaly

The potential energy anomaly is derived from the dynamic height anomaly by integration with respect to depth (Fofonoff, 1962). It is related to geostrophic transport in the same way that dynamic height is related to geostrophic currents. The potential energy anomaly was computed for stations along the Line (Fig. 30) and was used to describe the geostrophic transport across the Line. The potential energy anomaly is much more sensitive to changes of the density structure in the deep water than is the dynamic height anomaly. It can therefore be used in conjunction with the dynamic height as an integrated measure of the density distribution at a position.

Comparison of the contours of potential energy and dynamic height anomalies relative to the 1000-m surface (Fig. 30 and 27) reveals that the sequences throughout the period of observation were similar. This similarity is a reflection of the fact that the properties are consistent and the changes tend to be similar at all depths in the lower zone. As was the case for the dynamic height anomaly, the values of potential energy anomaly increased toward the coast (Station P to Station 1). Superimposed on this was an annual cycle in the eastern half of the Line as for the dynamic height. The highest values occurred from late summer to early winter (divergence season) and the lowest values from late winter to early summer (convergence season). The changes could be very rapid. Two cruises, carried out about a week apart during the beginning of August 1960, revealed a substantial change in the subsurface water. The potential energy anomaly increased about 2 units over the entire eastern half of the Line indicating a marked increase in the geostrophic transport.[5]

The average baroclinic transport across the Line cannot be estimated with precision because of the presence of wave-like variations of potential energy similar to those already noted in the anomaly of dynamic height. As these variations are transient and extend from the continental shelf to Station P and beyond, their contribution to the transport cannot be easily eliminated. In order to arrive at a representative value of the mean transport, the mean gradient of potential energy along the Line was determined by the method of least squares (Fig. 31). This method allowed values from all stations to be used and lessened the bias that would otherwise occur because of short-term fluctuations at individual stations, missing stations, and errors of measurements. The mean gradients of potential energy relative to selected standard depths from 300 to 2000 m for the 3-year period are given in Fig. 31.

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[5]The relative geostrophic transport of mass is given by the difference of potential energy anomaly between two points divided by the mean value of the Coriolis parameter. At the latitude of the Line, a difference of one unit (10^5 erg/cm^2) corresponds to a mass transport across the line of 0.9 million metric tons per second. Such a difference, relative to the 1000 m surface, would indicate that about 0.9×10^6 metric tons per second were flowing in the upper 1000 m relative to motion of the 1000-m surface.

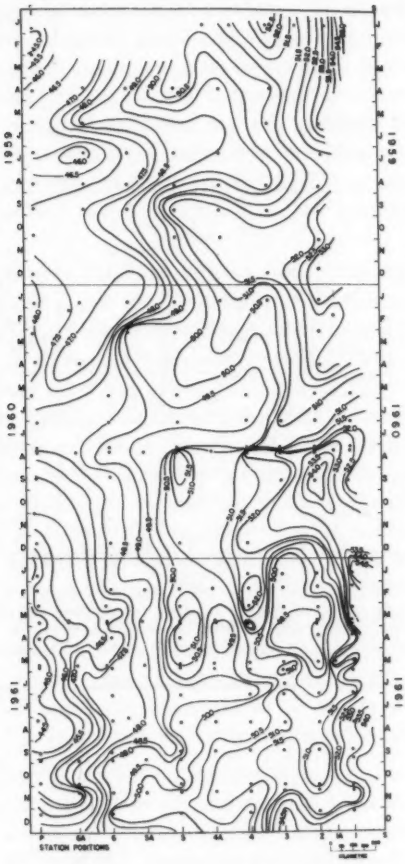

FIG. 30. The anomaly of potential energy (10^5 ergs/cm^2) relative to the 1000-m surface along the Line during the study period. Differences of the anomaly at a given time are proportional to the mass transport across the Line relative to the motion at 1000 m.

One of the more striking features of the mean gradients is the minimum reached during early summer of 1960. During this period, the subsurface temperatures at Station P reached a maximum while those near the coast fell to a minimum. No explanation was found for this behaviour in terms of variation of wind stress over the region.

The mean potential energy gradient along the Line for the study period is shown in Fig. 32. The curves were obtained by averaging the mean gradients from a number of cruises at each standard depth. The values to 1200 m are based on 30 or more cruises and to 2000 m on 18 or more cruises. Below 2000 m,

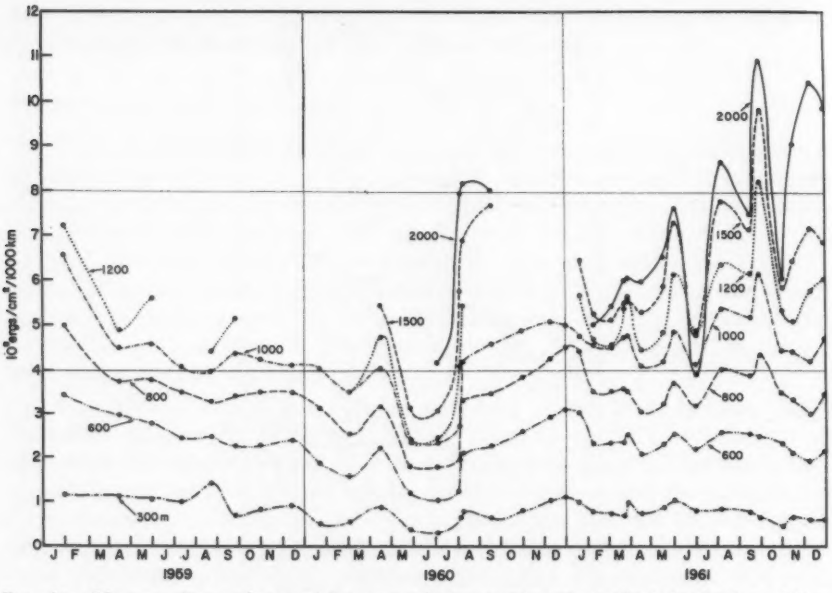

FIG. 31. Mean gradients of potential energy relative to selected standard depths from 300 to 2000 m for the study period. The gradients were estimated in terms of the mean difference in the potential energy over a distance of 1000 km.

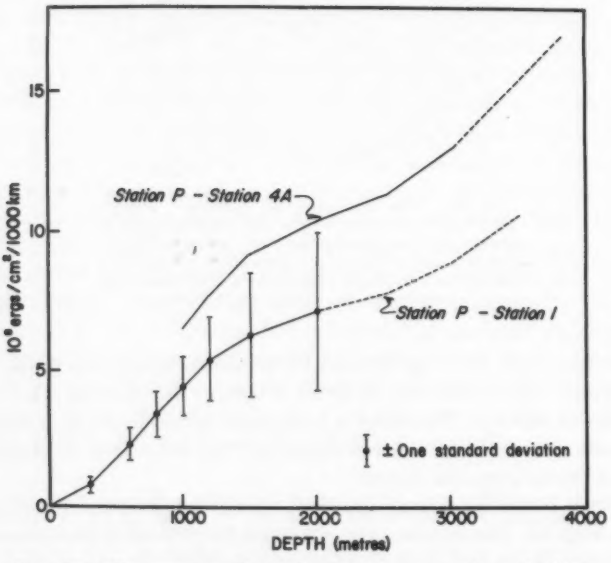

FIG. 32. The mean gradient of potential energy along the Line, 1959–61.

the points are averages of seven series of observations, six of which were made in 1961. The standard deviation about the 3-year mean is shown to the 2000-m depth. The second (upper) curve is the mean gradient in the western half of the Line based on the seven cruises for which deep-water observations are available. Where the curves overlap (1000–2000 m), the gradient in the western half of the Line is 46% greater than the mean for the Line as a whole. The mean curve for the Line was extrapolated for depths below 2000 m by assuming it to be in the same ratio to the western half as the gradient between 1000 and 2000 m.

The mean depth over the Line (Fig. 3) excluding the continental shelf is nearly 3500 m, and the mean gradient relative to this depth was 10.8×10^8 ergs/cm^{-2}/1000 km. The corresponding baroclinic transport was about 12.9×10^6 m^3/sec^{-1}. Half of this transport was contributed by the upper 1200 m of the section. An alternative estimate of the total baroclinic transport can be made by assuming that the gradient in the western half of the Line is on the average 46% greater than the gradient for the entire Line. Using the mean depths of 3800 m for the western half and 2800 m for the eastern half, the total transport was estimated to be 13.2×10^6 m^3/sec^{-1} or in close agreement with the former estimate. About 11×10^6 m^3/sec^{-1} was contributed by the western half of the Line and 2.2×10^6 m^3/sec^{-1} by the eastern half.

The mean total transport between 130° W and 145° W (Stations 3 to P), as computed from atmospheric pressure data, was 4.5×10^6 m^3/sec^{-1}. The corresponding estimate for the Line, adjusted for its greater length, was 5.8×10^6 m^3/sec^{-1}. If this value and the value for the total baroclinic transport of about 13×10^6 m^3/sec^{-1} are assumed to be representative, the barotropic transport must be 7.2×10^6 m^3/sec^{-1} directed southward across the Line.

The mean velocity profile[6] corresponding to the total flow (Fig. 33) indicates that the mean depth of no motion was 1400 m. The total northward flow above 1400 m was 7.4×10^6 m^3/sec^{-1} and the southward flow below 1400 m was 1.6×10^6 m^3/sec^{-1}, making a net northward flow of 5.8×10^6 m^3/sec^{-1}. The mean depth of no motion is not very sensitive to the magnitude of the total transport. It was calculated that the depth of no motion would lie between 1000 and 2000 m for all northward total transports between 0 and 10×10^6 m^3/sec^{-1}, for the potential energy gradient indicated in Fig. 32. As

[6]The mean velocity V(Z) is related to the mean potential energy gradient $\overline{\frac{\partial x}{\partial X}}$ (Z) (Fig. 32) by the geostrophic equation

$$\overline{\frac{\partial x}{\partial X}}(Z) = f\int_0^Z \rho[V(\zeta) - V(Z)]\, d\zeta$$

where f is the Coriolis parameter, ζ density of water, and Z depth. The equation is solved for the velocity profile V(Z) in the form

$$V(Z) = V_0 - \frac{1}{f}\int_0^Z \frac{1}{\rho\zeta}\frac{\partial}{\partial\zeta}\left(\overline{\frac{\partial x}{\partial X}}\right) d\zeta$$

and evaluated from the observed potential energy gradient. The integration constant V_0 is chosen to yield the total transport calculated from the curl of wind stress.

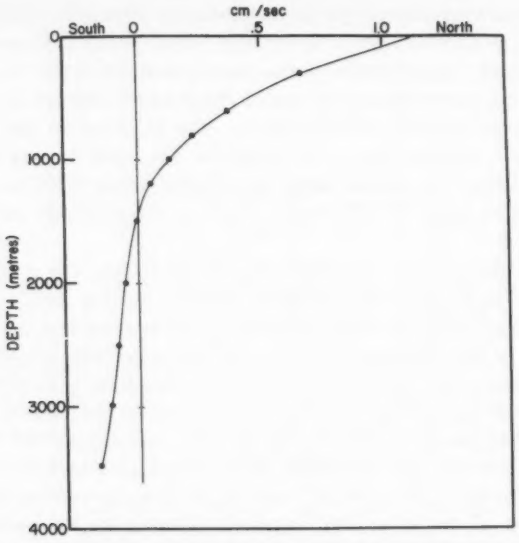

FIG. 33. Vertical profile of the mean geostrophic velocity assuming a total transport of 5.8 × 10^6 metric tons per second across the Line, 1959–61. (See text for derivation of velocity.)

the transport calculations based on means of atmospheric pressure probably underestimate the surface stress and, hence, the total transport because of the long averaging time used, the mean depth of no motion must be at least 1500 m along the Line.

CONCLUSIONS

The uniformity of the annual temperature cycle in the upper zone along the Line suggests that temperature structure is determined primarily by the local heat exchange and is controlled by meteorological factors. Currents in the ocean appear to have a relatively small influence on surface temperature.

The salinity in the surface layer is related to the discharge of fresh water from the land. However, the seaward extent and the persistence of diluted surface water offshore appears to be influenced considerably by the component of Ekman transport normal to the coast.

Variations of the depths of isopycnal surfaces in the vicinity of the continental shelf are related to the component of Ekman transport normal to the coast. However, the variation further to sea is related to the divergence of the Ekman transport, i.e. the curl of the surface wind stress. The relationship can be understood by assuming that the ocean responds barotropically to variations of annual period and shorter, and that the density field is advected by the barotropic motion.

Much of the observed behaviour of the ocean along the Line is not understood. The apparent presence of countercurrents, except along the continental slope, has no convincing explanation. The decrease of baroclinic transport to a minimum during the summer of 1960 and the subsequent marked increase (Fig. 31) does not correspond to any similar variation in the surface stress field. The real cause is not known with any degree of certainty. However, the decrease may be due to the presence of large-scale meander or eddy that is only partially intercepted by the Line.

Part of the difficulty in relating the behaviour of the ocean to variations in the boundary conditions can be attributed to insufficient data. Observations north and south of the Line are taken infrequently, usually not more than twice a year, so that conditions upstream and downstream from the Line cannot be described with any continuity. Along the Line, it is difficult to separate short-term variations from the more persistent changes because of the low rate of sampling in time. The deep water has not been sampled sufficiently frequently to delineate the changes in the density field that appear to occur. The station data provide no information on the barotropic motion and should be supplemented by current measurements in both the surface and subsurface layers.

Additional theoretical developments are necessary to establish the characteristics of the low-frequency response of the ocean in the presence of variable bottom topography and a continental slope. Further investigation of the interactions between barotropic and baroclinic modes of motion is required. Most theoretical studies (e.g. Veronis and Stommel, 1956) are confined to linear models in which no interaction terms are present.

ACKNOWLEDGMENTS

The collection of the oceanographic data along the Line involved several ships and many men. The authors are particularly grateful for the perseverance and patience of the seagoing scientists and technicians of the Pacific Oceanographic Group in collecting data throughout all seasons of the year, often under hostile weather conditions. The bulk of the data was collected from the weathership C.C.G.S. *St. Catharines*. The cooperation of Captain J. A. Sleight and the Officers and men is appreciated.

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Food and Growth of Fishes.
II. Effects of Food and Temperature on the Relation Between Metabolism and Body Weight[1]

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ABSTRACT

In an earlier paper we described the growth of fishes, $\Delta W/\Delta t$, in relation to the experimentally measurable variables, body weight (W), food intake (R), and total metabolism (T). Here we review experimental evidence of the nature of the relation between T and W, and its dependence on R and temperature. Making use of the basic energy equation, $pR = T + \Delta W/\Delta t$, where p is the term for correction from injested to utilizable energy, we calculate T as the difference between the energy equivalents of R and $\Delta W/\Delta t$, for comparison with results of oxygen consumption studies. Application to a number of published experimental results suggests that with constant food availability, this index of total metabolism, T, derived from feeding experiments, shows the same rate of change with body weight, W, as has been found by oxygen consumption studies under standard conditions. That is, the two sources of data provide estimates of a common γ in the relation

$$T = \alpha W^{\gamma}$$

where α and γ are the fitted parameters for the curve.

When fish are fed on a "maintenance" diet, the value of α calculated from the food-growth difference (the growth change is rarely nil in a given experimental observation period), appears to correspond with that characterizing the "routine" metabolic level in oxygen consumption studies. Higher α levels result from higher levels of food availability, and at *ad libitum* feeding α appears to approach the levels known in oxygen consumption studies as "active" metabolic levels. Temperature effects in the experiments were estimated from multiple regression analyses and showed an elevation of α with increasing temperature. The long-term effect of temperature on α was comparable with that predicted by the Krogh correction at *ad libitum* feeding, but was significantly lower when food was limited, as at "maintenance" feeding.

From a survey of effects of different designs of feeding experiments on these metabolic parameters, it appeared that apparently aberrant values of the weight exponent γ may instead be mistaken interpretations of changes in the level of metabolism α. That is, within the limiting conditions of standard or active metabolism, changes in temperature during experiments or manipulations of the availability of food by the experimenter, sometimes unintentionally, elicit adaptative responses in the level of metabolism, α. These show up in the results as effects on γ when the changes in conditions are gradual, hence confounded with body-size changes during growth.

The ability to make distinctions between effects of various factors on these two metabolic parameters appears to depend upon a distinction between experiments conducted with a view to learning what fish do under particular circumstances, and experiments designed to explore what fish are capable of doing. The former type reveal a remarkable conservatism in the basic relation between metabolism and body size, γ. Results from the latter reflect possibilities of metabolic adaptation to environmental circumstances. The apparent predictability of the response

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of the total metabolism to various conditions of food energy supply and dissipation suggests that the remainder of the energy system, represented by the growth, may be similarly predictable. If this is true outside the laboratory, measurements of the metabolic parameters, α and γ, already familiar in physiological and behavioural research, could be directly used as indices of the (relative) positions of various sizes and species of fish in natural production systems.

INTRODUCTION

THAT PART of natural production processes represented by growth of fishes has often been specified by a mathematical curve or equation fitted to observation points. The results have revealed a stability and small range of pattern among individuals and populations (Winsor, 1932a, b; Hjort et al., 1933; Brody, 1945; Baas-Becking, 1946; Smith, 1963). Yet such statistical fittings have not resulted in a knowledge of underlying relations which permits explanation of vagaries in the general pattern or extrapolation to new situations. In fact, as has been pointed out frequently in the literature, they are unlikely to do so (Gray, 1929; Kavanagh and Richards, 1934; Zucker et al., 1941a, b; Medawar, 1945). An alternative approach has been to postulate a basic mechanism and deduce a growth curve or pattern from it. The derived curves have been applied to data and, while they often fit the observation points, they too fail to explain deviations from the predicted pattern or to establish the validity of the postulated mechanisms (Richards, 1959; Hemmingsen, 1960; Taylor, 1962). It is apparent from such a record that for understanding production processes there is no adequate substitute for direct information on the underlying relations or mechanisms, such as might be provided by a combination of experimental and field observations.

The conceptual framework within which such direct information is to be obtained and interpreted has often been expressed in literature by the truism that all the input energy of the system must be accounted for. Considering growth as a part of an energy system, it is possible to write a general equation describing relations between energy input, its dissipation or expenditure in work, and its deposition as storage or growth. This starting point has been used, among others, by von Bertalanffy (1938, 1951), Lindeman (1942), Ivlev (1945), Patten (1959), McLaren (1963), and Slobodkin (1962), to describe the energetics of either whole trophic levels of the community complex, or of individuals in the system. However, the advantages of such simple formulation for the study of growth mechanisms have often been lost through failure to relate the terms of the equation to clearly measurable variables. For example, definition of net energy input and expenditure as anabolism and catabolism is to identify them with abstractions which cannot be unequivocally related to experimental variables (Brody, 1945; Steinbach and Moog, 1955). Similar difficulties have appeared in other attempts at detailed fragmentation of the basic energy system (Odum, 1956; Winberg, 1956).

Considering the dangers of abstraction to the success of production studies, Winberg (1956) proposed that at least initially, the basic energy equation for growth of individual fish be written in the form

$$pR = T + \Delta W/\Delta t \quad (1)$$

in which R is the rations consumed, p is a correction term for conversion of rations consumed to rations assimilated — in Brody's (1945) terminology the "physiologically useful" ration — T is the total metabolism, and ΔW the weight change, all terms being measured over a common period of time, Δt, and expressed in common energy units such as calories. Each term is here referable to an experimental variable. Thus, for most situations total oxygen consumption over an extended period of time may be taken to reflect the total release of energy in the fish body. It may therefore be equated to metabolism, T, without attempting to differentiate anabolic and catabolic components. Rations and weight changes may be measured directly, as may conversions to their energetic equivalents. Given the three measures with appropriate conversions, we have the three components of energy turnover which must balance, hence can be experimentally verified. Conversely, any one of them can be calculated if the other two are known.

This descriptive formulation clearly implies that to specify the functional process of growth and its regulation, we are required to link these components of energy flow with characteristics of the animals and the environment. That is, we are required to study relationships of T, R, and $\Delta W/\Delta t$, and their interactions, to some index of fish characteristics in different situations. Given such relationships, the growth can be described as an integral part of the production system as was demonstrated for a stable environment by an earlier paper (Paloheimo and Dickie, 1965). Here we review in more detail a number of the experimental studies of oxygen consumption, and the experimental studies of food consumption and growth on which our earlier formulation was based. Both types of experiments give valuable information on the relationship between metabolism and body size (weight), and the latter are especially valuable in showing how it is affected by food supply and temperature. Results of this study support our earlier hypothesis that for fishes, the basis for directly describing and understanding the natural interaction of rate of energy supply with its rate of dissipation is already well laid out. The simplicity and stability of the relations further suggest that the interaction of these components with $\Delta W/\Delta t$ may be similarly definable. The evidence for this will be examined in a later paper (Paloheimo and Dickie, 1966).

RESULTS OF MEASUREMENT OF OXYGEN CONSUMPTION

Relation Between Metabolism and Body Weight Under "Standard" Conditions

The relation between an index of metabolism of animals derived from measurements of oxygen consumption, and their body weight, is well known in the field of general physiology (Brody, 1945; Kleiber, 1947; Hemmingsen, 1960; Prosser and Brown, 1961). There are some broad differences between homoiothermal and poikilothermal animals. However, within each of these

major groupings there appears to be remarkable stability of pattern which is well described by the familiar equation

$$T = \alpha W^{\gamma};$$

or in its linear form (2)

$$\log T = \log \alpha + \gamma \log W$$

where T is total metabolism measured as volume (millilitres) of O_2 consumed per fish per hour (at standard temperature), W is body weight in grams, and "α" and "γ" are the fitted metabolic parameters. By reference to the more commonly used logarithmic equation, α is generally referred to as the "level of metabolism." The parameter, γ, describing the slope of the logarithmic equation is better known as the "weight exponent" and these terms are used here. In an extensive review of the literature and data for fishes, Winberg (1956, 1961) concluded that under standard conditions particular values for "α" and "γ" characterize almost all fish species. He found that at levels of metabolism close to the "standard" or "non-active,"[3] the exponent, γ, takes a value close to 0.8. Using the Krogh correction to adjust for temperature differences among different sets of data available to him, he further concluded that in most cases the data referred to levels above standard, which he called either resting or routine metabolism. At a body weight of 1 g and adjusting to 20 C, he found that an α value of about 0.3 appeared to characterize this metabolic level among fish from the temperate zone.

Winberg's value for the exponent for fishes is about the same as that cited by Fry (1957) for standard metabolism within fish species, and similar to that derived by others working with the same group of animals. For example, it is intermediate between the mean value determined on the basis of interspecific comparisons by Scholander et al. (1953) (0.85), and that deduced more recently by Hemmingsen (1960) from a series of interspecific comparisons (0.75). The only apparent exceptions to a value of this order of magnitude seem to be some of the common laboratory cyprinodonts (von Bertalanffy, 1951; Winberg, 1961), and the very young of some species (Winberg and Khartova, 1953),[4] although at least in the case of the young fishes there appear to be special difficulties in "standardizing" the condition of the fish at the time the observations are made (Winberg, 1961).

The data reviewed by both Winberg (1956) and Hemmingsen (1960) came from a wide variety of sources, representing work of different quality under a cacophony of conditions. Seeing order in the results required, besides

[3]The terms standard, resting, routine, and active, used to describe respiration experiments seem to be generally accepted and understood. For more detailed discussion and definition see Fry (1957).

[4]The apparently exceptional results of Keys (1931) and Wells (1935a, b) on *Fundulus parvipinnis* may be explained by vagaries of temperature effects and will be discussed below. Zeuthen (1947) draws attention to variability of determinations, and data for fishes in his fig. 32 and 40 support his interpretation that observations available to him were importantly influenced by factors other than body size.

careful criticism of methods of the original authors, transformations of much of their data, particularly for differences in experimental or ambient temperatures. This both authors accomplished by use of the Krogh (Ege and Krogh, 1914) temperature correction. Hemmingsen justified use of the Krogh correction on the basis that it reduced the scatter of the points about the fitted line. While noting the reduction in scatter, Winberg maintained that its use was perhaps best justified by the fact that it made possible his generalizations, despite some question as to the universality of its application to different species. As Job (1955) pointed out, its use in the study of the exponent γ of equation (2) can be justified only when there is assurance that the temperature effect is independent of size, an assurance which few investigators have been prepared to give unequivocally (Wells, 1935b). To use it in arriving at a definable level of metabolism (i.e. "resting" or "standard") is even more difficult, depending on many features of animal adaptation and the experimental conditions.

Some of the effects of temperature-related or other factors on the metabolic parameters derived from the respiration studies are reviewed here as a basis for our more detailed examinations of relations revealed by food–growth experiments.

Factors Affecting Estimation of the "Weight Exponent"

That the estimate of the exponent, γ, may be affected by complex interactions of metabolism and body size with temperature from season to season, or by adaptations to habitat temperature, may be illustrated by reference to the work of Barlow (1961). He found that the resting metabolism of fishes of the genus *Gillichthys*, which he collected and tested during "winter," showed a γ value of about 0.8 at 10 and 17 C, and a Q_{10} value close to that predicted by the Krogh "normal" curve. However, the same "winter" fish, when "acclimated" to 24 C (summer conditions?) and tested at 24 and 31 C, displayed γ values of the order of 0.5, the Q_{10} values being much higher for small than large fish. Barlow did not take differences in spontaneous activity into account, although it must be recognized that size difference may well involve differences in the activity response to temperature. In any case, the full meaning of the change in exponent in different parts of the temperature range cannot be assessed without similar information on fish in natural "summer" condition.

The work of Barlow is, however, of possibly great significance for the interpretation of the work of Keys (1931) and Wells (1935a, b). Both these investigators worked with *Fundulus* from the same area as studied by Barlow, and reported low exponents of the order of 0.5. The fish used by Keys were tested between 15 and 22 C, but most of the collections and O_2 consumption studies appeared to have been made during the winter months. Actual dates of collection and testing are not given, which now appears unfortunate, since his data (as plotted in his fig. 18) suggest the possibility of two groups of at least the smaller fish, and two possibly different exponent values as obtained by Barlow. This possibility is supported by the results of Wells (1935b),

who, while criticizing Keys' methods, also obtained a low exponent (ca. 0.50) for fish collected in winter and tested at 16 C. He obtained similar results with fish held at 10–12 C when they were tested at various higher temperatures (Wells, 1935a). But this is in marked contrast to the results he obtained in the same series of experiments when the fish were tested at their holding temperatures. We have calculated an average of his data for fish held and tested at 10–12 C and found a value of 0.79 for the exponent, close to that displayed by Barlow's fish in winter condition, and to the average value derived by Winberg. We conclude that temperature–season effects of this sort may play a determining role in the apparent value of the exponent. Similar effects may be involved in the seasonal differences in exponent noted for molluscs and Crustacea by Berg (1953), Berg and Ockelmann (1959), Rao and Bullock (1954), and for several species of fish by Zeisberger (1961).

Such varied results are sufficient to illustrate that the interactions of temperature and season with metabolism and body size may be complex, and that *a priori* transformations to "standard temperature" may not always be justified, at least in cases where fish have not been fully acclimated to test conditions, or where the distinction between routine or standard metabolism has not been carefully made. They may, of course, also indicate a lack of precision of present methods of oxygen determination.

The work of Graham (1949), Job (1955), Beamish (1964a), and Beamish and Mookherjii (1964) has been carried out with fish that are carefully acclimated to specified temperature regimes, and Beamish, at least, has taken careful account of activity changes. Their results suggest that, while some deviations from the Krogh correction curve may occur with particular species, such as brook trout,[5] the final effects of temperature are largely independent of size. That is, temperature–size interactions appear to result from differences in the rate of acclimation at different sizes rather than the end point of temperature effect. In the case of most species they examined, the Krogh correction appears to have been an adequate correction factor.

Beamish (1964a) reports the results of his experimental work on several species acclimated to different temperatures with effects of "spontaneous" activity eliminated, and compares them with results of other investigators. While there were some suggestive differences among species and temperatures in the exponent relating standard metabolism to body weight, few of these appeared to be statistically significant, and the influence of variations among individuals or due to small changes in experimental design cannot be eliminated. He concluded that the exponent for standard metabolism must be in the range 0.8–0.9, and compared this with other recently reported values of resting or routine metabolism of between about 0.7 and 0.9 (0.7 for *Gadus* sp.

[5]Job regards the Krogh correction factors as unsuitable for brook trout; however, the disagreement between Krogh's correction and effects of temperature measured from his own data is much less than is indicated in his fig. 4, where the Krogh correction line appears to have been incorrectly plotted. The correct Krogh line is indistinguishable in slope from that of Graham (1949), and does not seem to differ appreciably from Job's corrections at temperatures of about 15 C and less.

(Sundnes, 1957); 0.74 for *Squalus* and 0.78 for *Ophiodon* (Pritchard et al., 1958); 0.84–0.90 for various flounder species (Hickman, 1959)).

Similar values were reported by Wohlschlag and Juliano (1959) who measured oxygen consumption by bluegill sunfish in the field for specified degrees of activity. They found an exponent value of 0.83 for standard metabolism, except during early summer when they noted that a high value of 1.06 may have resulted from inclusion of several large spawning fish in the sample with smaller, immature individuals. Wohlschlag (1964) working with several species of antarctic fishes also found values for γ which departed considerably from an average value of 0.8. However, he concluded that these departures were in most cases the result of combining data for males and females which had different weight ranges and appeared to exhibit characteristically different levels of metabolism.

It can only be concluded that work with oxygen consumption of fishes indicates stability of the exponential-type relationship between metabolism and body size at both standard and routine metabolism. From a comparison of results of Winberg and other workers, it furthermore appears that the use of the Krogh correction for temperature does not introduce significant bias into comparisons unless possibly in the case of arctic and tropical species (Scholander et al., 1953, Wohlschlag, 1960) or where acclimation or seasonal metabolic phenomena are involved. A value for the exponent γ of about 0.80 seems to provide an adequate general description for this relationship among a majority of fishes under more or less constant, nonstress conditions.

Factors Affecting the Metabolic Level

By contrast with the apparent stability of the exponent, the level of metabolism expressed by α in equation (2) appears to be markedly affected by changes in a variety of conditions. Winberg (1956) after surveying the average level of metabolism he had calculated from the variety of experiments reported, not all of which were conducted under carefully controlled and specified conditions, concluded that his value for α of 0.3 ml O_2/hr at 20 C was somewhat higher than would characterize resting or standard metabolism and, following Job (1955), termed it the routine level. He suggested that it not only reflected elevation of metabolic level above standard under average nonstress conditions in the laboratory, but applied generally to nonstress conditions in nature. The successful use of the Krogh correction by Winberg and others for arriving at estimates of standard or routine metabolism implies that this correction provides a measure of temperature effects on α. However, it has been noted in addition that there are diurnal differences and effects of activity levels on α (Fry, 1957) as well as differences apparently associated with type of habitat (e.g. streams versus ponds (Winberg, 1956)). The latter observation especially suggests the possibility of adaptation of the level of metabolism to living conditions, an inference which has been drawn by other workers as well (Scholander et al., 1953; Bullock, 1955; Wohlschlag, 1960; Winberg, 1961).

The technical difficulties of defining some clearly repeatable level of standard metabolism have commanded considerable interest in themselves (Fry, 1957), but from the point of view of the study of growth, production processes, and community dynamics, the important feature is the demonstration that metabolic level is affected by conditions of both the fish and its environment. Thus, Beamish showed that many of the apparent changes in "standard" metabolism demonstrated by earlier workers were associated with changes in activity, although these were not simple. For example, he was able to show (Beamish, 1964c) that during starvation standard (i.e. nonactive) metabolism appeared to drop during the first 3 or 4 days but became constant thereafter. On the other hand, the initially higher routine metabolism, which included voluntary movement, dropped progressively during the long starvation periods, asymptotically approaching the standard level. He showed, however (Beamish, 1964b), that there are seasonal changes in the level of the nonactive metabolism, even in the absence of temperature or feeding effects, suggesting a basic change in body condition possibly associated with recognized hormone changes. Observations by Roberts (1964) on responses to light and temperature appear to have similar explanations. However, the influence of hormonal or some such fundamental adaptation is even more clearly indicated by Wohlschlag's (1962) finding of differences in metabolic level, associated with growth differences between the sexes of an antarctic species. There was no evidence to suggest that food intake differed between sexes. Saunders (1963) was able to show that the average level of total metabolism of codfish which were allowed to feed was considerably above that of nonfeeding fish, although there was no significant effect on the value of the exponent. The results of Job (1955) strongly suggest that in nonfeeding fish, changes in activity give rise to significant differences in level of metabolism, without significantly altering the exponent relating metabolism and body size, even when the fish of different sizes have all been stimulated to near maximum activity. Hemmingsen (1960) has gone so far as to suggest that the interspecific relation between active (i.e. maximum sustained) metabolism and body weight may have the same exponent as the interspecific standard metabolism, although the scattered experimental data available at present are hardly sufficient to provide a critical test of such an hypothesis.

Inferences from Oxygen Consumption Studies

It appears from these studies of oxygen consumption that there is among fishes the same basic conservatism in the type of relationship between metabolism and body size that has been noted within other animal groups. That is, the exponent γ relating metabolism to body weight in equation (2) appears to remain constant under a wide variety of conditions. However, the general level of metabolism defined in equation (2) by the coefficient α reacts in a rather complex fashion with changes in environmental and animal conditions. If constancy of the exponent should prove to be characteristic of the adaptation

of fish to given environmental conditions, this fact alone would provide significant simplification of the problem of understanding and studying the effects of factors underlying natural production processes. This possibility is further studied in the next section by reference to experiments on feeding and growth of fish.

RESULTS OF EXPERIMENTS ON THE FEEDING AND GROWTH OF FISHES

Use of Energy Equation (1)

Winberg (1956) attempted to check the validity of measures of energy dissipated during the growth of fishes by establishing agreement between results of direct oxygen consumption and those of the metabolic expenditure, calculated from the basic energy equation (equation 1) as the difference between food intake and growth. To make such comparisons it was, of course, necessary to introduce correction factors for conversions of experimental measurements into common units, and to determine a value for the overall food utilization coefficient which we have termed p in equation (1).[6] From his review of a number of studies he concluded that the two methods give essentially the same results.

From the point of view of study of growth and natural production processes there would appear to be several advantages in using the food–growth method of studying metabolism. In the first place, the relatively unrestricted holding conditions of a feeding experiment offer the possibility of clearer analogies to natural conditions than do the restricted conditions necessary during determinations of oxygen consumption. Secondly, in growth experiments the repeated observations of the same individuals over long periods of time provide data for detailed study of the contribution of individual and environmental variations to deviations from the average metabolic rate. Similar data could be obtained from direct respiration studies but this has rarely been the case in practice, partly because of the technical difficulties involved. Most important, however, the food–growth studies offer a method of directly measuring the effect of different availabilities of food and rates of feeding on metabolism, and growth, information which is essential to the study of production or growth processes. For these reasons we have reexamined the data from a number of feeding experiments in an attempt to clarify relations between the two experimental approaches.

Standardization of Measurements and Terminology

CONVERSION FACTORS FOR ENERGY EQUIVALENTS

Winberg (1956) examined the problem of converting measures of food, growth in weight, and oxygen consumption into common units such as calories.

[6]Descriptive terms for "p" become awkward, as is apparent in the general literature as well as here. Some discussion of its meaning and measurement is given below. It is not to be confused with such deceptively simple terms as "digestibility" coefficient.

There is no doubt of the desirability of having directly determined conversion factors, although these have very rarely been supplied. Thus, one would hesitate to rely too heavily on results from long-term feeding experiments in which there is clear possibility of fluctuation in caloric content per unit weight of fish flesh or food, such as might normally be expected from season to season or place to place, unless these were carefully measured. However, Winberg found that a number of studies purporting to derive accurate conversion factors have frequently introduced new errors when they attempted to eliminate others. He concluded that in the face of seasonal, geographic, and species fluctuations, approximate conversion factors may often be preferable, a point of view which seems supported by stability of the overall average of measurements of caloric content of a variety of whole animals by Slobodkin and Richman (1961) and Golley (1961). In what follows we have adopted Winberg's approximate conversions where direct estimates are not available. That is, we assume that consumption of 1 ml of O_2 represents release of about 5 cal of energy, and that since dry weight of animal tissue contains on the average about 5 g cal/mg, the consumption of 1 ml O_2 is equivalent to the expenditure of 1 mg of dry body substance. Such conversions permit easy and useful order of magnitude comparisons of general level of metabolism from the two types of experiments.

In very few of the experiments we reexamined have direct determinations of caloric values of food and fish flesh been reported, so that it has been necessary to make certain assumptions. An assumption of constancy of relative caloric content of food and flesh may not be seriously in error in cases where the quality of the food has not knowingly been changed, although initial variability in some experiments (Dawes, 1930a, b) suggests a need to be especially critical of results immediately following introduction of a new feeding regime, new type of food, or new holding condition. Strict equality of caloric values seems rarely likely to be a valid assumption and corresponding adjustments in the metabolic equivalents have been made wherever possible. Finally, while nutrient values of natural foods may generally be adequate, this has not often been verified. The pathological results of prolonged experimental feeding on deficient diets or those containing possibly cumulative toxic substances, as observed by Brown (1951), are dramatic warning. Effects of these sorts may therefore influence metabolism measures and must certainly contribute to variability in the experimental results. In reviewing past data we can do little to correct for such effects beyond exercising caution in interpretations, especially in making interspecies comparisons.

THE "PHYSIOLOGICALLY USEFUL" RATION

It is recognized that not all of the consumed ration actually enters into the metabolic system of the animal. A certain fraction is passed out directly in the faeces, and another excreted directly in the urine. From his examination of the evidence, Winberg concluded that the coefficient p of R was approximately equal to 0.8. This value is undoubtedly subject to variation depending

on the kinds and quantities of foods consumed, and the feeding conditions (Ricker, 1946; Gerking, 1952; Menzel, 1960; Davies, 1963). However, Ivlev (1960), who studied the physiology of food utilization in fishes as exhaustively as anyone, concurred with Winberg that it is a useful approximation. The following considerations lead us to support this view for studies at least of the exponent γ, provided the true value is not in fact very much less than 0.8.

To elaborate: It is apparent from consideration of equation (1) that changes in the degree of food utilization will be indistinguishable from the differences in relative energy values: both influence the value of the coefficient p in the equation. Moreover, a change in p will result in a comparable change in T, hence the estimate of the level of metabolism, expressed by α of equation (2) is directly affected. In addition, it can be seen from equation (1) that for a particular value of $\Delta W/\Delta t$, a given value of the coefficient p will have a different relative effect on the magnitude of T at different levels of the rations, R. Since R and W will frequently be correlated, a possibly serious consequence is that the value of p can have an influence on the estimate of the exponent γ of equation (2).

Fortunately, it appears that with relative values of R and $\Delta W/\Delta t$ of the order found in most data on fish even large errors in p would be unlikely to introduce appreciable error into estimates of γ. This may be inferred directly if we write the balanced energy equation in the form

$$T = pR - \Delta W/\Delta t.$$

If the efficiency of utilization of food for growth, $K = \dfrac{\Delta W}{pR\Delta t}$, stays almost constant with increasing R, we may rewrite the above equation as

$$T = \alpha W^{\gamma} = \frac{\Delta W}{\Delta t}\left(\frac{p}{K} - 1\right).$$

From this approximation it appears that a change in the coefficient p will primarily affect the value of α, rather than γ. In a study reported later (Paloheimo and Dickie, 1966), we find that K does depend on R and W. However, the dependence is small, so the foregoing conclusions appear to hold. As a further check, we have employed different trial values for p in calculating values for T from equation (1), using the data on "maximum" feeding and growth of brown trout from 2.8 to 85.2 g reported by Pentelow (1939). The results with respect to the interpretation of the trout data are examined in more detail later. Here we need only report that changing the value of p over the range 0.6–1.0 gave estimates of γ ranging from 0.86 ± 0.06 to 0.85 ± 0.06; at lower values of p, the estimate of γ increased more rapidly. Hence, we conclude that a value of the utilization coefficient, p, of the order of 0.8, derived by Winberg from a combination of biochemical, physiological, and ecological considerations, can be accepted as a convenient working value for general

studies of metabolism, with some assurance that we will not thereby significantly bias estimates of the exponent relating rate of change of metabolism to body weight.

Aside from effects of changes in p on the exponent, any errors in assigning relative nutrient, energy, or assimilation values to food and growth will, through their effect on p, alter the estimates of level of metabolism α almost in proportion. Where we have the requisite information we may, from the above results, consider appropriate corrections for metabolic level independent of their relatively minor influence on the exponent.

COMPARISON OF EXPERIMENTAL CONDITIONS

The problem of establishing the equivalence of the terminology and the experimental conditions used in oxygen consumption and feeding studies is more difficult than the problem of deriving conversion factors and utilization coefficients reviewed above. In certain cases a solution to this third problem is tantamount to defining the basic concepts underlying particular studies of metabolic phenomena. For example in respiration experiments frequent reference is made to measurements of the standard, resting, routine, or active metabolism. In feeding experiments, on the other hand, workers have subjected their animals to "maintenance," "restricted," "proportional," or *ad libitum* (i.e. unrestricted) diets. An assessment of the degree of similarity in the measures of metabolism resulting from some of these usages may be made by reference to results of two familiar sets of experiments with the brown trout, *Salmo trutta*, reported by Pentelow (1939) and Brown (1946b). Other comparisons are made at later stages in our analysis.

Pentelow reports the results of an experiment in which fish of different initial sizes were fed a "maintenance" ration. That is, the food supply was continually adjusted so that in the long run the individual fish neither gained nor lost weight. Assuming initially that the caloric value of the weight of the food (live *Gammarus pulex*) was approximately the same as that of the trout, we have used equation (1) to calculate metabolic expenditures in terms of live body weights. No attempt was made by Pentelow to control water temperatures, which were allowed to vary in approximately the same manner as did temperatures in nearby streams which were natural habitat for the trout. However, Pentelow gives an average temperature for each experimental observation period of 3–7 weeks (low of 3.5 C, high 19.5 C, average 8.9 C). Rather than assume the applicability of the Krogh correction for temperature to a situation where we know nothing of the degree of acclimation or seasonal state on metabolism, we have preferred to estimate a correction term empirically by assuming a multiple regression equation of the form:

$$\log T = \log \alpha + \gamma \log W + (t^\circ - t_0) \log Q_{01} \qquad (3)$$

where α and γ should be numerically identical with the constants of equation (2), t° is the observed temperature measured from some standard reference

value, t_0, and Q_{01} is the temperature correction/1 C appropriate to an assumed log–linear relationship with metabolism. Q_{01} may be compared with the conventional Q_{10} used as a measure of the average correction for a 10 C degree temperature change. Over most of the temperature range of the experiments reviewed here, the Krogh correction does not differ much from a linear logarithmic effect, thus some appreciation of its appropriateness can be obtained from the estimate of Q_{01} in this form.

The calculations for Pentelow's trout results in an estimate of $\gamma = 0.79 \pm 0.07$, which is in agreement with the values cited earlier for other species on the basis of measurements of O_2 consumption. It does not agree with Pentelow's own conclusions which were that the metabolism of a trout was proportional to its weight. However, he did not attempt a statistical analysis of his data, and seems to have based his conclusions partly on a summary table (his table VII) which, because it gives average values for very wide size ranges of fish, does not seem appropriate as a basis for study of the relationship, and partly on the simple relation between this average body weight and average metabolism, neglecting temperature differences. When we omitted the temperature term from our calculations (i.e. use equation (2) rather than (3)), the resulting "simple" slope from Pentelow's results was $\gamma = 0.87 \pm 0.09$.[7] Effects of temperature revealed by feeding experiments will be reviewed later. For present purposes, it seems safe to conclude from the value of γ, that these "maintenance" feeding experiments described by Pentelow yield an index of the same metabolic phenomena as have been studied by measurements of oxygen consumption.

The calculations of equation (3) for Pentelow's data yield a value for the "level of metabolism" intercept (i.e. the metabolism at a weight of 1 g) of $\alpha = 0.109$ g wet body weight per week at the average temperature of 8.9 C. This is equivalent to about 22 mg dry body substance. Winberg expresses his basic equation for metabolic rate at a standard of 20 C. Correcting our calculated value to 20 C on the basis of the multiple regression and expressing the results on a per hour basis yields a value for $\alpha = 0.26$ mg dry body substance or ml O_2 per hour, with lower and upper confidence limits of approximately 0.22 and 0.30. The value therefore overlaps with the central value of 0.30 suggested by Winberg as characterizing the routine metabolic level of fishes. If our initial assumption that the food value of the live *Gammarus pulex* per unit volume was equal to that of the fish is incorrect but, as is more likely, that the food contained fewer calories per unit volume (Karzinkin, 1952), then we have probably overestimated the level of T for the trout. However, from the close correspondence between the values for both slope and positions of the metabolism–body size relation, it seems safe to conclude that the condition described as a maintenance feeding regime was imposing on the fish approximately the same condition as had been described in respiratory work as the condition of routine metabolism.

[7]Since no temperature correction is mentioned, it appears that Horton's (1961) estimate of $\gamma = 0.84$, based on some apparently unpublished data of Pentelow, corresponds with this estimate based on the published data, but neglecting temperature effects.

Brown (1946b) has demonstrated, however, that trout may be acclimated to various levels of apparently "maintenance" feeding. That is, if a fish be fed for a time on a ration on which it maintains its body weight, and the food supply is then slightly curtailed, it begins to lose weight. This weight loss may continue for periods of up to 2 weeks, but if the new rations level is above a certain minimum, the weight of the fish will again become stabilized. If then, the feeding rate be increased slightly, body weight increases for a short time before the fish becomes acclimated to the new feeding level and weight again becomes stabilized. Brown carried out a series of experiments under constant temperature with trout of between about 30 and 120 g in weight, progressively curtailing their food supply with the object of determining the "minimum maintenance" level. Her results, when plotted in logarithmic form for comparison with Pentelow's results yield a somewhat irregular line with an estimate of $\gamma = 0.35$ (approximately).

This low value of γ from Brown's experiments indicates that large fish were better able to adapt to the stress of a drop in supply of food than were the small fish, since there was a markedly greater downward displacement of their metabolism when food supply was curtailed. We have little basis for measuring the actual amount of this adjustment since no caloric values are given for the "raw meat" diet used as food during the experiment; that is, we have no estimate for α (equation (2)). But the magnitude of this enforced adaptation was considerable and the differences in the degree of adjustment over the size range are in keeping with observations of survival times of different-sized fish under starvation conditions (Ivlev, 1961).

The difference between the relation of body size to the *minimum* maintenance requirement, revealed by Brown's experiments, and the "no growth," hence maintenance, metabolic expenditure revealed by the Pentelow experiments, indicates that it is not always a simple matter to extrapolate from the equilibrium conditions of minimum maintenance to other states, a point stressed by Brody (1945, p. 42). In fact, these experiments of Brown and Pentelow seem to be studying two fundamentally different phenomena. From the work of Beamish (1964c), it seems likely that one of the factors involved must be the degree to which voluntary activity by different sized fish can be changed during periods of food restriction. Even more basic may be the fact that in the Pentelow-type experiment the rate of food-administration is determined by the "demands" of the fish, whereas in the Brown-type the fish is required to adapt to new conditions set by the experimenter. Further discussion is delayed until we have more data assembled below. Whatever the difference, the important fact for present purposes is that comparison of these two sets of results with those from respiratory work emphasizes that the feeding regime may have a critical bearing on the results of metabolic measurements, and must be considered in attempts to interpret them or to understand their significance in the natural environment. With this stipulation, we may with some justification conclude that estimates of metabolic expenditure derived from maintenance feeding experiments of the type described

by Pentelow correspond with estimates of routine metabolism derived from respiratory work. This reference point permits us to proceed with a further study of results of feeding experiments.

Effect of Level of Feeding on Metabolism

Studies of the physiological processes underlying individual growth have commonly posited that there exists a basic maintenance metabolic requirement which must be satisfied by food energy intake *before* energy is available for other uses. Thus, the feeding experiments of Pentelow, and before him, of Dawes (1930a, b), were conducted with a view to establishing a maintenance food requirement which could be subtracted from the total food consumption before studying net growth efficiency and the factors influencing it. A similar conception of energy relations is outlined by Brown (1957), and is implied by the growth models of von Bertalanffy or of Beverton and Holt (1957). However, in addition to the fact that any such "average" correction must increase the relative variability of the derived metabolic indices, Brown's work (1946b) showing acclimation of the apparent equilibrium maintenance metabolic requirements to different feeding levels, Beamish's observations on the importance of modification of activity levels on the routine metabolism under different conditions, and especially with starvation, together with Saunders' (1963) recognition of a change in the level of metabolism in fed and starved animals, strongly suggest that the concept of a fixed "maintenance" metabolism is, for fishes, an abstraction of doubtful value in the understanding of natural growth energetics. A similar point of view was expressed much earlier in the study of metabolism and feeding of warm-blooded animals by Forbes et al. (1934). In fact, there does not seem to be any necessity of assuming a fixed maintenance requirement. Equation (1) provides a means for studying the effects of changes in feeding level on the total metabolism under various conditions without any such *a priori* dissections of the system.

Pentelow's trout

Pentelow's (1939) results illustrate the effects of changes in the level of feeding on total metabolism. In a set of experiments under roughly the same conditions as described for his experiments on maintenance rations, he held trout in separate tanks and allowed them to feed *ad libitum*; that is, he maintained an excess of the food, live *Gammarus*, in the tanks. Records were kept of the amounts of food put into the tanks. Periodically they were cleaned and the remaining amounts of food determined. His tables supply records of fish sizes, calculated food consumption, and growths of individual fish over 3- to 7-week intervals for periods of up to $1\frac{1}{2}$ years, together with "average" temperatures during each observation period. As was done for maintenance rations, we have used the energy equation (1) to calculate the index of metabolism, T, in terms of wet weight, from these *ad libitum* feeding data, and to examine its relation to body size and temperature. The results of both sets of calculations are plotted in logarithmic form in Fig. 1. The estimates of T plotted in the

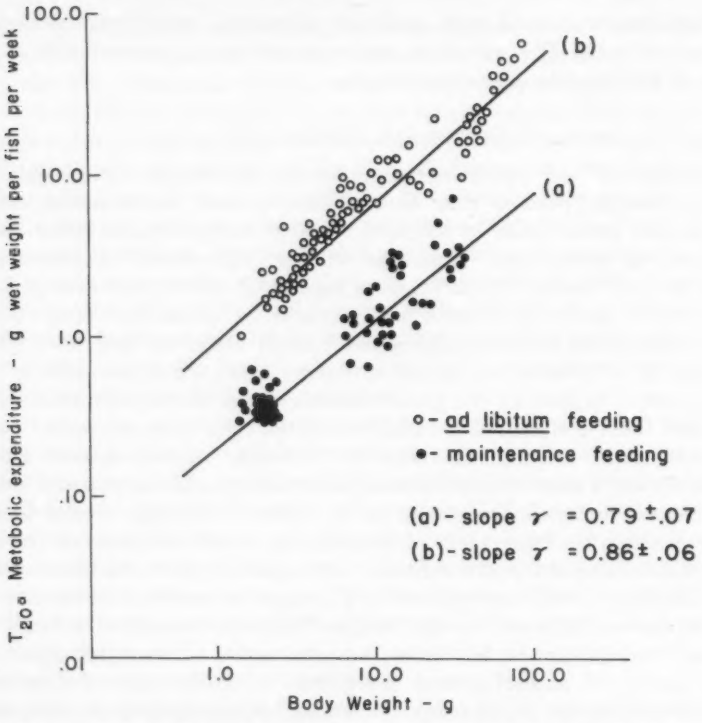

FIG. 1. Relation between body weight and total metabolism in individual brown trout at different rates of feeding (data from Pentelow, 1939); line (a) at maintenance feeding, line (b) at *ad libitum* feeding. Metabolism was calculated from Winberg's energy equation ($T = 0.8\ R - \Delta W/\Delta t$) assuming the fish and rations have the same calorie content per unit weight. Temperature adjustment was made from the multiple regression equation assuming a log–linear effect (see equation (3)).

figure have all been corrected to 20 C on the basis of temperature effects shown empirically by the experimental data (i.e. calculated from equation (3)).

There is a marked difference between the positions of the two lines, the average level of T for the *ad libitum* feedings being about five times that for the maintenance ration. This difference is comparable in magnitude to the difference between standard and active metabolism determined by Job (1955) for brook trout, and cited for other species as well (Fry, 1957; Brett, 1962), on the basis of oxygen consumption. The slope of the *ad libitum* feeding line, which we have calculated for the brown trout appears to be slightly greater than that for maintenance feeding, but the difference is not statistically significant, the slope of both being indistinguishable from the average value for γ suggested earlier. It thus appears that the effect of increasing the level of availability of food to the trout was a large increase in the level of metabolism, α, without affecting the relationship between metabolism and body size, γ.

DAWES' PLAICE

A comparable phenomenon is revealed by our reanalysis of the data from Dawes' (1930a, b) experiments on the feeding of 2-year-old plaice (*Pleuronectes platessa*). For the most part, Dawes maintained individual fish in cages at ambient surface water temperatures for periods of several months each. These fish were fed on chunks of freshly caught mussel (*Mytilus edulis*) flesh. One series was kept on a maintenance ration, another fed *ad libitum*, and a third fed on "intermediate" or restricted rations. Dawes calculated food consumptions and weight and length changes at approximately 2-week intervals, and gives detailed records, together with initial weights. Average temperatures are given in separate graphs. We have used these records, converted to a standard 2-week period, to calculate metabolic expenditures, T, again in terms of changes in weights of wet body substance, assuming as with Pentelow's data that relative caloric value of food per unit volume was constant throughout at a value of 1. The resulting estimates of metabolic level, α, could perhaps have been adjusted to nearer the actual by making use of the observations of Hatanaka et al. (1956b) that the ratio of caloric content of freshly shucked clams to Japanese plaice flesh was about 0.7:1. However, we are primarily interested in the relative values at different feeding levels. Since most of the experiments at different feeding levels were run simultaneously, it seems justifiable to assume that any seasonal or other caloric changes that may have taken place were parallel. We have thus avoided making the superfluous and possibly inapplicable caloric corrections.

The regression lines relating metabolic expenditures, T, to body weight, W, for male and female plaice were not significantly different. Lines for combined sexes have therefore been calculated and shown in Fig. 2, again corrected to 20 C by the multiple regression temperature coefficient (equation (3)) to facilitate comparisons. This figure shows that lines for plaice on maintenance rations and for those on *ad libitum* rations are again parallel, with the latter markedly displaced upwards. The difference indicates that the level of metabolism for active feeding was about four and one half times the maintenance level. As might be expected, the estimated metabolic expenditure for the "intermediate" ration is between these two (about twice the maintenance level). A notable feature of the curves for plaice, compared with those for other species, is that for both maintenance and *ad libitum* feeding levels the slopes, γ, are low, the data providing estimates of $\gamma = 0.63 \pm 0.06$ and 0.62 ± 0.06, respectively, significantly lower than γ values for most other species, and also lower than the γ for intermediate rations (0.96 ± 0.20). This low γ, which may have resulted from interactions of the metabolism–body size relationship with temperature and the design of the experiments, will be discussed in more detail below.

KINNE'S DESERT PUPFISH

Other feeding experiments are reported in the literature, and the results of at least some of them confirm the general effects of feeding suggested by the Pentelow and Dawes experiments. Among the most detailed and carefully

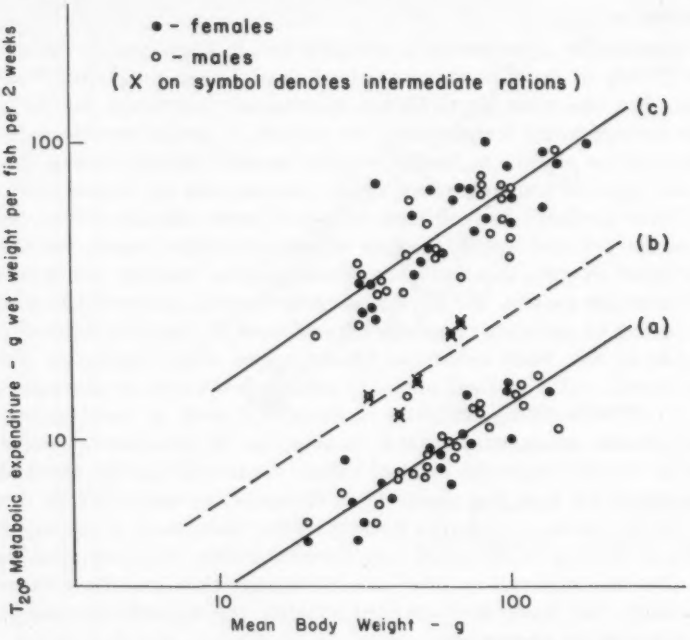

FIG. 2. Metabolism–body weight relations of North Sea plaice at 20 C at 3 different levels of feeding: line (a) at maintenance feeding, line (b) on "intermediate" rations, line (c) at *ad libitum* feeding (data from Dawes 1930a, b). Plotted points are the means for individuals or for a group reared together, and lines (a) and (c) are the corresponding partial regression lines, all corrected for temperature differences as in Fig. 1.

conducted of these are the feeding experiments of Kinne (1960, 1962) (largely carried out in Bullock's laboratory where Barlow also worked) on the small euryplastic "desert" pupfish of southern California, *Cyprinodon macularius*. Groups of 6–12 fish were kept at different constant temperatures and salinities and fed a diet of live *Enchytraeus albidus*, supplemented occasionally with fresh lettuce and spinach. Biweekly records of average size, food, growth, and growth efficiency are given for each group of fish when fed on a "restricted" supply of food. This regime is described as follows: Fish were fed daily, except Saturday and Sunday, until each fish of the group was satiated, then 50% more food was put into the tank; the extra food was normally eaten during the next few hours. An unrestricted food schedule was apparently tried briefly but due to technical difficulties was not continued and not reported in detail.

We have again calculated metabolic expenditures and their relation to body weight using Kinne's records for saltwater rearing in terms of dry weights (Kinne's measure). (Conversion from the length data supplied in the paper to weight was done using tables made available by Dr Kinne.) The results are plotted in Fig. 3, each point representing an average value for the 6–12 fish.

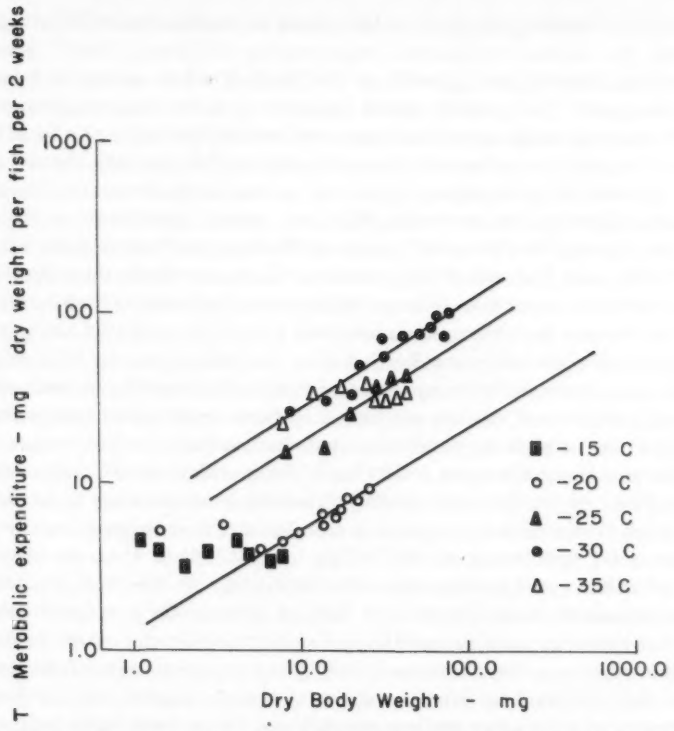

FIG. 3. Metabolism–body size relations in groups of the desert pupfish, fed to satiation +50% daily, at five different temperatures (data from Kinne 1960, 1962).

They show that at 15 C there was no difference in average metabolism of the different small sizes of fish involved. The same level of metabolism appeared to hold for small fish at the 20 C temperature. However, at a dry weight greater than about 7 mg (living weight equivalent of 27 mg — Kinne, personal communication), metabolism showed the expected increase with body size at temperatures of 20, 25, and 30 C. At a temperature of 35 C, metabolism at first rose, then dropped sharply for the larger fish. Kinne concluded from various observations that the restricted supply of food was limiting growth at 35 C more seriously than at the others; the metabolic drop may perhaps be similarly interpreted. Eliminating these possibly atypical data for small fish and for 35 C, we have calculated the regression of metabolism on body size for the 20, 25, and 30 C temperature groups, adjusting for differences in the mean level among them by covariance.

The resulting common slope, γ, within temperature groups is 0.63 ± 0.10, which, while lower than that cited as typical of most fishes, is the same as that for the other exceptional cyprinodont fishes according to von Bertalanffy

and found by Winberg, both from his review of the literature (Winberg, 1956), and later by special respiration experiments (Winberg, 1961). Kinne also observed the feeding and growth of the pupfish when reared in fresh water rather than salt. The growth, hence range of sizes for these experiments, was small, relative to the usual variations in rations. Our estimate of $\gamma = 0.35 \pm 0.18$ is lower than that found in salt water, suggesting that the adjustment to living in low salinities may represent an extra metabolic load for the smaller fish.

With respect to the level of metabolism, results of Kinne's experiments in salt water also confirm the conclusions of Winberg and before him, Scholander et al. (1953) and Bullock (1955), while at the same time they illustrate the effects under discussion here. That is, fishes normally living at high temperatures appear to have a low level of metabolism when measured at some standard and compared with fish normally living at low temperatures. Winberg (1961) concluded on the basis of comparison of results reported by Scholander et al. (1953) with reports of various studies of Chinese and Indian freshwater fishes normally living at high temperatures, that the routine metabolic rate of warm-water fish was about the same at 30 C as is the metabolism of "temperate zone" fishes at 20 C. From Kinne's results, *Cyprinodon macularius*, which normally lives at high temperatures, displays a metabolic rate of approximately 0.09 ml O_2/hr for a 1-g fish living at 20 C. This is only half of the rate shown by a variety of other cyprinodonts tested by Winberg. At 25–30 C, however, the index of metabolism of *Cyprinodon* fed on intermediate rations was about 0.30, or 1.5 times the routine metabolism characterizing the other cyprinodonts at that temperature. Despite some uncertainty about the exact relative caloric value of the fish food, it is thus safe to conclude that Kinne's experiments illustrate the phenomenon among warm-water fishes that with feeding there was an elevation in level of metabolism above the routine, without significant alteration of the slope characterizing the relationship.

OTHER SPECIES

Results of other feeding experiments, while insufficient for detailed study, confirm the relationships in a general way. For example, Brown's (1946b) experimental results show high variability which may partly reflect difficulties of accurate measurement of food consumption with the feeding of ground meat and liver. However, the data on food consumptions and growth per unit weight for restricted and *ad libitum* feeding of brown trout have been read from her graphs, and fish weights estimated from her graph of maintenance requirements. From the data so obtained, metabolic expenditures were calculated from the energy equation. The results indicate a higher average metabolic level at the higher feeding rates, confirming our results from Pentelow's experiments on the same species.

Ivlev (1939) calculated the nitrogen balance for a group of young carp grown in a pond in which the food consisted almost entirely of chironomid larvae. Using his data, and correcting for differences in temperature by using Krogh's correction, we have calculated a value of $\gamma = 0.85$ and of $\alpha = 0.8$ ml

O_2/hr at 1 g. This value for α is about three times the routine level deduced by Winberg.

The experiments of Gerking (1952) on bluegill sunfish (*Lepomis*) carried out over a 50-day period at an average temperature of 25 C suggest a γ of about 0.8, although the differences between individual fish are great. Gerking (1955b) himself estimated from experimental measures of nitrogen excretion that the γ for this species must be low, of the order of 0.54 over the weight range 0–170 g. However, his fig. 2 (p. 287) shows that this estimate depends heavily on the one point for a fish larger than 110 g. When it is eliminated, the slope is indistinguishable from 0.8, because of individual variability, hence agrees with results obtained from measurements of oxygen consumption by Wohlschlag and Juliano (1959) on the same species. Other experiments were conducted by Gerking (1955a) in which food was administered at different rates. In these experiments the size range is too small to permit us to check the slope of the metabolism–body size relation at any one feeding level, but there is a clear indication of an increase in α with each increase in amount fed, the level at full feeding being about four times the maintenance level.

Analysis of covariance of Menzel's (1960) data on feeding and growth of a warm-water Bermuda reef fish suggests a value of $\gamma = 0.76$ within temperature groups with an α of about 0.3 mg dry wt/hr at an average temperature of about 23 C, that is, two to three times the routine level for warm-water fishes.

Data of Baldwin (1956) at 9, 13, and 17 C on *ad libitum* feeding and growth of the brook trout (*Salvelinus fontinalis*) on live minnows gave an estimate of γ within temperature groups of 0.72 ± 0.17 when data for a large fish which developed tail rot were excluded from the calculations, and Surber's (1935) data for the same species fed *ad libitum* on *Gammarus* at a temperature of about 12 C gave an estimate of $\gamma = 0.56 \pm 0.22$, when we included the possibly aberrantly high first observation period out of the five successive observation periods reported. In both cases, our estimate of α calculated from these data on wet body substance corresponds with estimates for active metabolism of the brook trout based on oxygen consumption measurements by Job (1955) when, for comparison, his results are interpolated to 13 C. The three sets of data for the brook trout are plotted in Fig. 4. A value of $\gamma = 0.80$ was also derived from Surber's data for similar experiments on the rainbow trout (*Salmo irridens*), the level of metabolism, α, being indistinguishable from that characterizing the active level for the brook trout.

The interpretation of other food–growth experiments is questionable. The data for the Japanese plaice, *Limanda yokahama*, given by Hatanaka et al. (1956a) show a slope $\gamma = 0.65 \pm 0.28$ within temperatures, but the limited size range of fish used, together with the high variability, does not permit us to make meaningful estimates of the level of metabolism. In a number of other feeding experiments the small range of body sizes and high variability of results, sometimes perhaps because of varying temperature, does not permit accurate estimation of either γ or α. We have been unable to use the data of Hatanaka et al. (1956b) on the plaice, *Kareius bicoloratus*, or of Kohler (1964)

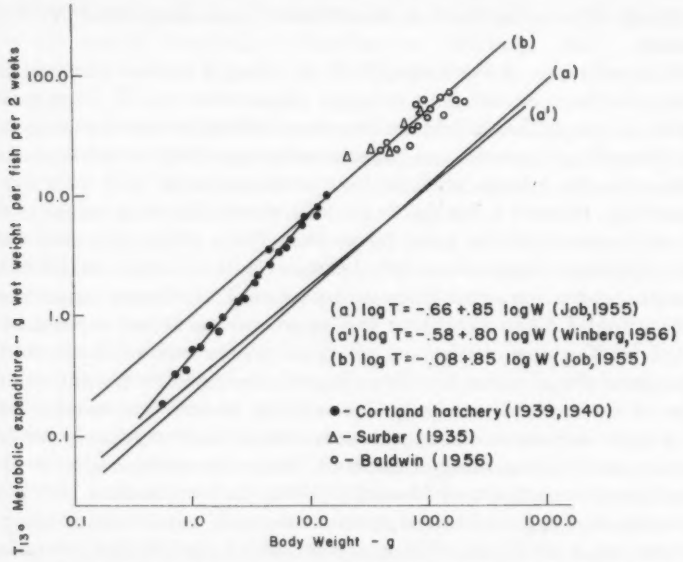

FIG. 4. Metabolism–body size relations in brook trout at 13 C. The Cortland hatchery fish were fed on hatchery mash in large groups in proportion to body weight. Surber fed a group of four fish *ad libitum* on *Gammarus*; Baldwin fed individual fish on live minnows. Lines (a) and (b) represent standard and active levels of metabolism interpolated to 13 C from the data on O_2 consumption by Job (1955). Line (a^1) is the line for "routine" metabolism of all fishes given by Winberg, but corrected to 13 C using the Krogh correction.

on the cod (*Gadus morhua*), on this account. In addition, we have rejected the results of Markus (1932) on the largemouth bass, partly because his material seemed to consist of selected fast-growing fish, but primarily because on one occasion the reported growth in weight exceeded weight of food intake, and on at least one other occasion was an inordinately high proportion. No explanations were offered. Records describing results of pond-rearing of large numbers of the loach by Kubota (1961) were also discarded since on at least one occasion they showed growth equivalent to more than 90% of the gross food consumption, and on a number of occasions growth of over 60% of the ration. We regarded these as inordinately high values and in the absence of other special explanations concluded that problems of feed accumulation, delayed consumption, and wastage, such as have been encountered in hatchery-feeding experiments (McCay and Tunison, 1934, 1935), meant that food–growth measurements cited by Kubota could not be assigned with sufficient accuracy to particular observation periods.

SUMMARY OF FOOD–GROWTH EFFECTS

The results of our re-examination of the above set of experimental data offer strong support for Winberg's conclusion that data on growth and feeding yield

estimates of the metabolism which show the same basic relationship with body weight, γ, as has been found in extensive researches of oxygen consumption. In addition, it appears, despite some uncertainty about the precise conversion factors for different kinds of food and fish flesh, that when fish are fed at different rates on the same food there is a direct effect on the level of metabolism, α. This varied from a level close to that defined as "standard" metabolism in oxygen consumption studies when fish were fed a maintenance diet, to close to the maximum sustained active level when they were fed an *ad libitum* diet. Levels of food supply intermediate between these extremes apparently give rise to metabolism–size relations of a similar sort at an intermediate level of metabolism. Finally, we observed that indications of the same phenomenon were reported for several different species of fish, fed different kinds of food, and under rather different holding and temperature conditions.

Two further sets of data from carefully executed sets of feeding and growth experiments with brook trout have been examined. These were conducted at the Cortland Hatchery and used a relatively wide range of fish sizes. The results, discussed in the next section, provide some contrasts with the results described above, and at the same time furnish a better basis for understanding the mechanisms which may underlie the relationships.

EFFECTS OF DIFFERENCES IN THE FEEDING REGIME

In the foregoing we have considered mainly the differences in metabolic level characterizing different "planes of nutrition" (Brody, 1945), each defined by a particular type of feeding plan or schedule. In one, the maintenance diet, feeding rate was adjusted by the experimenter in accordance with the response of the weight of the fish; in "restricted" diets fish were fed to satiation at a predetermined frequency; in the *ad libitum* diet, the criterion for determining amount fed was that there should always be a small amount of food in the tank in excess of the fish's consumption of food. All these systems have in common the fact that the amount fed was adjusted to take explicit account of the demand shown by the fish. While there was a different level of metabolism for the different feeding levels, these systems of adjusting feeding to fish demands resulted in a common relation of metabolism to body size, evidenced by an apparent parallelism of the slopes within species for all feeding levels (i.e. a common γ).

A contrast to this result was provided by the work of Brown (1946b, p. 27), in which fish were forced to adapt to as small a ration as possible. The resulting lower slope of the metabolism–body size relation suggested that the interaction of the feeding regime and the adaptability of the fish may have an important bearing on metabolic measurements and their interpretation.

Another type of fish-feeding system has been practiced in fish-rearing and experimental work. A study of the results gives further information on the effects of the feeding regime and its significance. In this system, the amount fed is kept at a constant proportion of the weight of fish to be fed. The system works well for short experiments but, if experiments are begun with small

fish and the experimental period is long enough that the growth is considerable, it frequently happens that the food supply dictated by the weight gradually comes to exceed the amount which the fish is able to consume (Gerking, 1955a). That is, an *a priori* weight-proportional feeding system of this sort, rather than responding to the demands of the fish, is presenting fish, as in Brown's experiments, with a set of changing conditions to which they may or may not adjust. Adjustment appears to continue until the level of maximum intake is reached, at which point the system breaks down. To avoid undesirable accumulation of food in the tanks, the feeding schedule must be changed and becomes indistinguishable from *ad libitum* feeding.

The practice of feeding in proportion to body weight was common in earlier hatchery work and is the system described in much of the work by the Cortland Hatchery. As pointed out by McCay and Tunison (1934), in the early work at the hatchery the initial proportion of food to weight of fish was frequently high, so that food may have accumulated in the tanks during some feeding periods and been eaten during subsequent periods. The early experiments also encountered the opposite difficulty: that of ensuring that the weight of food administered was eaten by the fish and not lost in solution. However, part of these difficulties seems to have been eliminated from later work, and we have used results of two series of feeding experiments on the brook trout (*Salvelinus fontinalis*) reported in Cortland Hatchery reports for 1939 and 1940 for this study (Tunison et al., 1939; Phillips et al., 1940).

In both experiments large numbers (more than 1000) of small fish were introduced into tanks maintained at a constant temperature of 13 C. Each tank was supplied with an amount of food per day equivalent to about 8% of the initial weight of the fish. At 2-week intervals a sample of fish was weighed, and enough individuals then removed from the tank to bring the calculated total weight of fish in it back to the starting point, while the rate of feeding was held constant. (The removed fish could be weighed, measured, and examined for body constituents and water content.) In the experiments considered here, a number of different diets were tried in parallel experiments conducted over periods of 24–36 weeks each. Our statistical tests revealed no differences in the metabolism between diets within years or between years. But because of a slight difference in fish sizes between years, average values of fish sizes, food consumptions, and growths were calculated by 2-week periods for each year's experiments separately. Phillips et al. (1949) further note that although the constitution of the body of trout in terms of protein, ash, fat, and water content may change with diet and age, there is no appreciable change in these factors for the range of weights and ages involved in these experiments. We have therefore calculated the index of metabolic expenditures in terms of live weight of trout flesh.

The results of the metabolic calculations for the brook trout from the Cortland Hatchery data and for the results given by Baldwin (1956) and Surber (1935) are plotted together in Fig. 4. We also show estimates of metabolic relations from the respiratory work by Job (1955) and for all fish according to

the Winberg equation, converted to terms of equivalent expenditure of live body weight. These estimates of metabolism are all adjusted to 13 C, the temperature at which the Cortland Hatchery experiments were made. Throughout the hatchery experiments the metabolism of the trout was clearly above the standard metabolic level determined by Job (1955) and, except at the beginning, it was above that deduced by Winberg for average routine metabolism. However, it increased relatively steeply during the course of the experiment, and at the end approximated the active level. The average slope of the regression (i.e. γ) is about 1.19, although there is some indication of a levelling off at the largest sizes or highest food consumptions. The data, which are averages for hundreds of fish, show very little deviation from the line, the error of estimate of the average slope being only ± 0.01.

There are no other experiments known to us which provide comparable data on the metabolic rate when feeding is proportional to body size. Gerking (1955a) fed his fish certain different proportions of body weight as a basis for defining different levels of feeding and at higher feeding rates ran into the problem of the rate of supply becoming excessive. However, his experiments were conducted over relatively short periods of time (50 days), involving relatively little weight change, and he gives only initial and final weights for two fish at each feeding level. While the slopes of metabolism on body size indicated by his data are suggestively high, with so few closely spaced points at each level, but signs of considerable fish-to-fish variability, we did not consider the data to provide reliable confirmation of the phenomenon exhibited by the Cortland Hatchery data.

The Dawes plaice experiments described earlier yielded estimates of γ which were the same at maintenance and maximum feeding levels, but higher at intermediate feeding levels. Dawes (1930a) pointed out in his first set of feeding experiments that food for intermediate feedings was held at about one-third the amount being taken by the maximum-fed fish. Since these intermediate feedings were resulting in less growth than did maximum rations, it is evident that the ratio of food to body size must have increased relatively faster. The possibly higher slope for metabolism is therefore suggestive of the type of phenomenon exhibited by the Cortland Hatchery trout work.

These results giving high slopes for the relation between metabolism and body size at intermediate rations are in contrast to the results obtained from our analysis of Kinne's (1960, 1962) and possibly also of Hatanaka's (1956a) data. They, too, dealt with restricted, or intermediate rations. But their intermediate ration resulted in a slope for the metabolism–size relation which seemed to be parallel to that characteristic of routine metabolism. However, unlike the *a priori* feeding system for the hatchery trout, Kinne's fish were fed to satiation once a day, then an extra ration was allowed them. Hatanaka's were fed to satiation once a day. These systems might be considered comparable with the *ad libitum* feeding system in the sense that the fish were permitted to eat more or less according to their demands although for a restricted period of time. That is, the systems of Kinne and Hatanaka limited the feeding time

rather than specifying *a priori* the amount of food. That restricted feeding periods lead to characteristic food consumptions below those dictated by maximum stomach filling has been demonstrated by the work of Rozin and Mayer (1964).

It is clear that the definitions of regime by which we are able to characterize various feeding experiments are not as precise as we might wish. Nevertheless, the distinctions we can make leave little doubt that changes in the feeding system are responsible for apparent alterations in γ, the relationship between metabolism and body size. The limited results available suggest that the extent of the alteration in γ may reflect the extent to which changes in the amount of food available elicit adaptive responses in the rate of food consumed.

Such adaptations in feeding and metabolism would be possible only between the normal limits characterizing full feeding or active, and maintenance feeding or routine metabolism. At both these limiting levels, the consumption of food by the fish appears just sufficient to maintain it in a relative dynamic metabolic state characteristic of fish of that body size. Kinne's and Hatanaka's data suggest, moreover, that a similar relationship is to be expected at intermediate feeding levels. That is, at intermediate rates of feeding, γ should be the same as it is at the limiting levels, provided that the design of the experiment has maintained a constant availability of food so that the results reflect the animal's food demand under constant conditions rather than its capacity to adjust to new conditions. With such few results, however, this conclusion requires confirmation in experiments designed specifically to test it.

SOME INTERACTIONS OF TEMPERATURE AND FEEDING ON METABOLISM

Reference has been made above to some of the complicating effects of temperature on metabolic indices. An exhaustive review of this vast field of study is outside the scope of this paper. However, in the experiments reviewed here, temperature variations have affected the results of the food–growth experiments in a manner which requires description, if not explanation.

In addition to the general elevating effects on metabolism of increasing temperatures within the biokinetic range, it has long been known that there are differences in the immediate and long-term responses of metabolic indices to temperature (Grainger, 1958) as well as differences in the rate of adjustment to temperature increases or decreases (Fry, 1947; Brett, 1956). In the face of this, it is at least enigmatic to find that the Krogh temperature correction, first based on the immediate response of O_2 consumption of a single goldfish to temperature changes (Ege and Krogh, 1914) should be found to apply to temperature effects measured in rather elaborate careful experiments on fishes fully acclimated to different holding temperatures (Beamish, 1964a). It is less surprising that the correction may not hold for some species (Job, 1955), for other physiological functions or activities (Fry and Hart, 1948; McLeese and Wilder, 1958), or for fishes from extreme environmental conditions (Wohlschlag,

1960). In view of these complexities we have preferred where possible in our analyses to assess and correct for temperature effects on the basis of the experimental data (i.e. equation (3)) rather than to use *a priori* correction factors. The results, while not simplifying our knowledge of temperature effects, perhaps permit a better evaluation of the significance of some of the experimental results; although in such attempts to let the data speak for themselves there is the obvious danger that metabolic effects assigned to temperature are actually the result of confounding with weight and ration changes, or that there is significant curvilinearity which was not taken into account by our assumed log–linear relation. These general points are illustrated by reference to further details of our reanalysis of the food–growth data.

Effects of Temperature Change on the Relation Between Metabolism and Body Size

It was noted in reviewing effects of level of feeding on metabolism that although the temperature "corrected" metabolism for Dawes' plaice showed a marked rise in level with increase in food, the rate of change of metabolism with weight, γ, appeared to be significantly lower for both maintenance and *ad libitum* feeding than it was for most other species. However, to accept this observation as characterizing the species is to assume that during the experiments the degree of acclimation to the changing experimental temperatures or temperature correlated factors was comparable at all sizes, and was uniform over all parts of the season. Results of earlier investigations of temperature effects make it seem likely that both these assumptions may have been violated. Furthermore, in the experimental design the temperature effects have been confounded with size to an unknown degree, fish of various sizes having been started at different times during the season and observed for different periods of time. It is of major importance to interpretation of the results to measure the influence of these effects.

Some of the details of our reanalysis of the plaice data are given in Table I. As a first step, the overall observations which we have used to derive the slopes for the multiple regression lines of Fig. 2 were adjusted by covariance analysis for differences in mean values for individual fish. Since the spread of fish sizes used in the experiments was considerably greater than the size change due to growth of individuals, this resulted in a reduction in the range of size and of metabolism. This presumably contributed to the generally lower precision of the estimate of $\gamma = b_{Y,X_1/X_2}$ within individuals in Part I. However, for maximum feeding levels there appeared to be a lower slope within individuals than that shown by the overall data. On the other hand, the slopes drawn through the means for each individual appeared to be higher than those within individuals. Such a divergence of the slopes within individuals from that between means for individuals suggested interactions peculiar to the experimental procedures. At least a partial explanation has been sought in differences in the temperature histories of the individuals.

TABLE I. Regression analysis of Dawes' plaice data showing partial regression coefficients, b_1 and b_2, and their errors where Y = log T (metabolism), X_1 = log W (weight), $X_2 = t°$ (average temperature). A fourth variable, condition factor, was also included in the analysis, but had an insignificant effect and is therefore omitted from the table. Results for males and females were not significantly different so that the table is given for combined sexes. b_1 is the partial regression $Y,X_1/X_2$ and b_2 is for $Y,X_2/X_1$.

	Level of feeding								
	Maximum (*ad libitum*)			Intermediate			Maintenance		
Temperature series:	A+D	A	D	A+D	A	D	A+D	A	D
Part I: Partial regression coefficients $b_1 \pm 2\sigma_{b_1}$									
Overall data	.62 ± .06	.46 ± .10	.76 ± .10	.96 ± .20	0[a]	.64 ± .35	.63 ± .06	.65 ± .10	.63 ± .07
Within individuals	.49 ± .08	.52 ± .30	.67 ± .20	.93 ± .25	0	0	0	1.60 ± 1.40	0
Individual means	.66 ± .15	.47 ± .15	.77 ± .20	.61 ± .08	0	0	.62 ± .10	.65 ± .11	.57 ± .12
Part II: Partial regression coefficients $b_2 \pm 2\sigma_{b_2}$									
Overall data	.031 ± .005	.034 ± .006	.025 ± .007	-	-	-	.010 ± .003	.006 ± .005	.015 ± .005
Within individuals	.022 ± .005	.020 ± .018	.031 ± .007	-	-	-	.014 ± .005	0	.024 ± .005
Individual means	.037 ± .013	.035 ± .009	0	-	-	-	0	.007 ± .005	0

A = ascending temperature series
D = descending temperature series
A+D = overall series
[a]By the value 0 we mean that the standard error of the estimate was so great that the average value calculated from the data was not distinguishable from 0 slope at the 95% probability level.

Individual plaice were begun in the experiments at different times with respect to the temperature maximum. Accordingly, we have divided the data for each individual into two parts, an ascending temperature series which begins at the end of the observation period during which the seasonal minimum temperature occurred, and ends with the period during which the temperature reached its seasonal maximum; and the remainder defined as a descending temperature series. The multiple regression analyses and covariance adjustments were then carried out separately on each of the ascending and descending temperature series. The results in Table I, Part I, show that for the *ad libitum* feeding the overall slopes are significantly lower for the ascending temperature series than they are for the descending temperature series, hence the mean for the whole series apparently depended on the relative lengths of time fishes were observed in each part of it. The same phenomenon is apparent within individuals and for the individual means although in these cases precision of the estimation is low. That is, the slopes in all cases depend heavily on the distributions of sizes of fish and observation periods throughout the temperature series. The data are not therefore sufficient to demonstrate whether or not plaice have a metabolism–size relationship different from other species, although the data, especially for means of individuals give little reason for supposing the species is an exceptional one of the Cyprinodontiformes type. Fortunately, in view of the complexity of interaction with temperature, the maximum and maintenance feeding series which we have used in this review were run in parallel during the experiments. The similarity of slopes may therefore be taken to represent similarity of reaction to experimental conditions and justify assigning changes in level of metabolism to feeding effects even where we cannot use them to establish "standard" levels.

Data for the intermediate feeding levels are insufficient for determination of the interaction with seasonal temperature. However, the high estimate of overall γ for the intermediate ration appears to be related to the "within" individual effects, since the slope of individual means is of the order of that for other feeding levels (see Fig. 3). Since in the intermediate feeding experiments two rather different temperature series were involved for the small number of different fish observed, this result supports our earlier suggestion that the high overall slope may reflect the gradual changes in "intermediate" feeding level within individuals rather than temperature changes.

Despite the variability within the ascending and descending temperature series, the data suggest that during rising temperatures the metabolic rate of smaller individuals was higher, relative to that of larger fish, than it was during the descending temperature series. Such a situation might arise when in an ascending series the small individuals "adjusted" faster to the higher temperatures than did the larger fish. At least the latter case receives support from the fact that those larger plaice exposed to temperatures higher than 20 C showed a marked decrease in growth rate, whereas the smaller plaice seemed not to be adversely affected. It appears then that the relatively faster metabolic

rise of the smaller fish is an adaptative response, whereas the limited response by the larger fish represents limited adaptability rather than rapid acclimation.

Distinctions of this sort are of interest in connection with observations of patterns of distribution of fishes with seasonal temperature changes in nature. For example, it is generally found that adult winter flounders (*Pseudopleuronectes americanus*) move from deep into shallow water in spring and spawn there, while the surface waters are still relatively cool. As the water warms, they move into deeper, cooler water while the young remain and grow in the warm shallows (McCracken, 1963). However, as the young fish age and grow they too tend to perform longer seasonal migrations which eventually have the effect of keeping their environmental temperatures from the extremes encountered by the young. Similar phenomena have been noted for cod (Jean, 1964). From the experimental results with plaice it appears that this habit is in keeping with a limited ability of the larger fish to adapt to temperature changes of the type encountered in shallow water. These size-dependent differences in adaptability are reminiscent of similar changes in the scope for metabolic activity of trout of different sizes found by Job (1955).

In view of these results with Dawes' plaice data, it is of interest that despite seasonal temperature changes Pentelow's data for trout yielded a slope so nearly like that of other fish. Our analysis of Pentelow's data in two temperature series similar to those used for plaice, showed that, while there are differences in means of the ascending and descending temperature series suggestive of the type encountered in the plaice, these differences were not significant. This result was obtained despite the fact that several of the trout were reared and observed through nearly two annual temperature cycles. The temperature changes in Pentelow's experiments were generally smaller and slower than in Dawes' experiments, making it more likely that the rates of acclimation to temperatures by all sizes of the trout were more nearly the same than they were for plaice. In any case, the design of the Pentelow experiments does not appear to have biassed the resulting measures of metabolic relations as seriously as in Dawes' experiments.

Interaction of Food and Temperature

The plaice and trout data show a further, rather striking effect of temperature. That is, the partial regression coefficient for the assumed log–linear temperature effect on metabolism ($b_{Y,X_2/X_1}$) (note that this term is identical with the term Q_{01} of equation (3) when temperatures are measured in degrees centigrade beginning at $t_0 = 0$ C) is lower for the maintenance feeding level than it is for the *ad libitum* feeding regime. For the plaice, Table I, Part II, shows that the overall temperature effect at maintenance feeding was 0.010 ± 0.003, while at *ad libitum* feeding it was 0.031 ± 0.005, at average temperatures of about 15 C in each case. Comparable estimates for the trout data were 0.028 ± 0.007 and 0.055 ± 0.005 at average temperatures of about 9 C. The effects for both species are comparable to the effects predicted by the Krogh

corrections for the maximum feeding level while at maintenance feeding they are only about half the Krogh correction.

This observation would appear to be consistent with the results expected from an interaction of effects of feeding and temperature on metabolism. That is, starting at a low level of temperature and feeding, an increase in temperature alone may be expected to give rise to a higher metabolic level, part of which may be expected to result in higher voluntary activity ordinarily resulting in long-term higher food intake. If, however, the food supply should be manipulated in such a way that there is a restriction on the possible increase in food consumption, as during feeding on maintenance rations, the final metabolic level should reflect the downward acclimation of total energy exchange of the sort studied by Brown (1946b) in response to this restriction. However, if food were available in excess, as in the *ad libitum* feeding experiments, one would expect the increased activity to give rise to an increase in food consumption. To the long-term metabolic response to temperature must then be added the sorts of response noted earlier to a change in food supply alone. The result to be expected in such a case is an apparently greater effect of temperature when food consumption is allowed to vary in response to the animal's demands, than when food consumption is restricted.

This interpretation may be compared with the results of Kinne's experiments. There, the rate of feeding was restricted only in the sense that the total daily food intake was limited to the consumption of a fraction of what it might have been under an *ad libitum* regime. At each temperature, however, the fish were fed 50% more than required to satiate them in one daily feeding. From the foregoing explanation of interactions of temperature and feeding, it might therefore be expected that the overall food restriction in Kinne's experiments may not have affected the activity responses or changes in metabolic level with temperature in the same way as an *a priori* restriction on total food available. From Kinne's results we have calculated that for this warm-water species the average regression coefficient for the log-linear temperature effect between 25 and 30 C was about 0.04. This result is to be compared with the Krogh correction index for temperate zone species between 15 and 20 C (Winberg, 1961), which is also 0.04. While no data are available for maintenance rations, this correspondence is in keeping with the results and interpretations of the plaice and trout experiments.

Kinne's results also show, however, that caution must be exercised in interpretation of the order of magnitude of apparent temperature effects measured by the multiple-regression technique. That is, these data reveal a temperature coefficient of nearly 0.10 between 20 and 25 C, indicating that the log-linear temperature effect between 25 and 30 C does not apply over the lower part of the temperature range investigated. Data on plaice and trout are too variable to provide a critical test of the assumption of linearity over different parts of the range. Nevertheless, the differences in mean temperature effect for the whole series were in both cases highly significant statistically, suggesting a strong effect of both food supply and temperature on metabolism.

DISCUSSION

The foregoing review confirms that there exists the same remarkable sort of conservatism of the relationship between metabolism and body size in fishes as is found in other animal groups. This relationship, as reflected in γ of equation (2), which was first demonstrated by studies of oxygen consumption under defined states of rest, voluntary movement, or sustained activity, in various environmental and body conditions, appears again in the metabolic indices derived from the balanced energy equation (1) applied to growth and feeding experiments. Results of these experiments further demonstrate that this basic relationship reflected in equation (2) by the exponent, γ, appears under different stable conditions of food availability and temperature. That is, although the basic level of turnover of energy in the fish body changes with changes in the rate and conditions of supply and use, adjustments of the physiology and behaviour to a new situation, involving possibly complex interaction of components of resting and active metabolism with the food energy supply, appear to lead to the establishment and maintenance of particular relative metabolic levels with respect to body size. This conservatism of the basic response implies some underlying feedback or homeostatic mechanism operating on the dynamic energy system.

The existence of homeostatic mechanisms as an important feature of regulation of physiological processes has been recognized for many years in warm-blooded animals where body temperature is maintained within narrow limits despite body size and food intake changes. It has become recognized only recently that the relationship must rest on a wider base involving not only complex interaction of the physiological processes of the animal, but including interactions with its behaviour, or what is often described as "voluntary" or "spontaneous" activity as well. Thus, Cottle and Carlson (1954) showed a "voluntary" increase in food consumption by rats to meet the increased caloric output required by exposure to low temperature; the rates of adjustment and the effect of temperature varied with size. Hamilton (1959) demonstrated that when rats are exposed to cold, those kept on a low diet manipulate a switch to operate a heat lamp for longer periods than those on full diets. Edholm et al. (1955) found compensatory responses with a significant lag period in the food intake of army cadets following varying levels of activity indicating, even in cerebral man, the apparently unconscious or automatic nature of the integration of physiological with behavioural responses which achieve homeostasis.

That the homeostatic responses of intake of particular foods are linked with the level of essential nutrients in the body was indicated earlier by Mayer-Gross and Walker (1946) who found that the palatability of various concentrations of sugar depended on blood sugar levels, and that sugar substitutes indistinguished from sugars by normal individuals were rejected by hypoglycemic individuals as not "sweet." Thus, although voluntary food intake is related to palatability, palatability is itself importantly related to nutritional condition (Lepkovsky, 1948). These facts were dramatically underlined by the work of

Keys et al. (1950) in their report of the Minnesota experiments in human starvation.

Such evidence leaves little room for doubt that warm-blooded animals have an ability to adjust their feeding habits and levels of intake towards the maintenance of a nutritional state to which they have become acclimated. That the same is true of fishes is illustrated by experiments of Rozin and Mayer (1961, 1964). They trained goldfish to push a lever in order to obtain pelleted food. After the fish appeared to have established a particular level of feeding, in terms of numbers of pellets injested per unit time, the nutrient value of the pellets was changed. The fish compensated within a day by changing the rate of lever pressing and pellet ingestion, maintaining their established nutrient intake level despite a twofold change in bulk ingestion. Similar compensatory variations in food ingestion were demonstrated in response to change in temperature. Rozin and Mayer also showed that different stable levels of food intake were established by the fish, depending on the length of time of their opportunities for feeding, and on the "effort" necessary in order to get the food.

The mechanism responsible for the homeostatic control of nutrient state while apparently specific (Mayer, 1955) is not instantaneous, although it appeared to be more rapid in the case of goldfish than has generally been found in warm-blooded animals (Janowitz and Hollander, 1955). Neither, as pointed out by Broboek (1960) with reference to response of food intake to temperature, can the mechanism controlling voluntary food intake and energy balance be simply related to the rate of metabolism. It must involve both the energy intake and energy output (Grossman, 1955). This is supported for fishes by our findings that the effect of temperature on general metabolic level was different at different levels of feeding and possibly in the ascending and descending temperature series as well. It would appear, in fact, that rather than being able to isolate factors of priority in energy equation (1), we are dealing with part of a circular causal system (Frank, 1948; Hutchinson, 1948) working to maintain a specific type of dynamic balance in the animal body. That is, total energy expenditure, T, results in a certain component which may be termed active metabolism, giving rise to activity which in its turn acts on food supply to produce a new physiologically useful ration, pR, which supplies the metabolism. Such a circular causal system will lead to new equilibrium states for a change in any part of the system provided the "gain" from rations to metabolic level is less than unity and the lag period is short enough to produce damping of oscillations (McCulloch, 1948).

We must conclude then, that the apparent stability of γ under various constant conditions of food supply and temperature implies a feedback system, operating through the integration of physiological and behavioural mechanisms, to maintain a particular dynamic state out of the range of possible states which could have been deduced logically by reference to our starting point of energy equation (1). Given appropriate conditions, the experimental results thus reflect the adjustments that fish of different sizes *do* make to their environment, rather than their *capacity* to react. By contrast, apparent changes in γ under changing

temperature and feeding regimes may often reflect the capacity of the fish for adaptations or acclimations towards reestablishment of new equilibrium states. The newly established states must however satisfy the same criteria, apparently related to nutrient state, which underlie the regular phenomena revealed under stable conditions. Since temperature and feeding conditions must in many cases be the most important of the variables to which fish must adapt in nature, it follows that the regulation of living conditions in this manner must have a determining influence on the condition of the dynamic energy system which result in natural biological production in the form of fish metabolism and growth; hence our use of the relationship in formulation of growth equations (Paloheimo and Dickie, 1965).

It is apparent, however, that, while the general functional form of the relationship between metabolism and body weight is well supported, and that the experimental evidence points strongly to a value of γ of about 0.8 under natural conditions, we still know very little about the corresponding levels of metabolism. That is, we cannot from the experimental evidence reviewed here establish the form of the relationship between the level of metabolism, α, and the ration, R. From the data presented here we do know that the total range of possible values must lie within that already defined as standard and active metabolism by studies of oxygen consumption. Winberg (1956) concluded that in normally feeding fishes it is intermediate between these two levels — of the order of 1.5–2.0 times the standard rate. Further discussion of the relationships has to be deferred until we examine the growth in relation to metabolism (Paloheimo and Dickie, 1966).

An inquiry into the conditions under which metabolic level is stable or will change is a vast field for further experimental study. For example, response of metabolic level to temperature has been well described, yet certain of the experimental results reviewed above suggest that in the long term the expression of this effect may well depend on feeding conditions. It has also been observed that when fish of some species are placed in groups there is a lowering of the metabolic level (Schuett, 1933; Delco and Beyers, 1963), although similar effects have not been observed in others (Bowen, 1932; Wells, 1935a). Such effects may be mediated through the influence of group behaviour on activity. But whatever the explanation, such observations suggest the possibility of effects of density on growth other than those resulting from a simple enforced sharing of the food supply. In addition, it will be noted that in all the food–growth experiments examined here the size of fish observed in a tank at any one time was more or less uniform. While with growth this permits examination of the response of different sizes of fish to a standard feeding regime, we cannot be assured that the presence of competition from different species or different sizes of the same species will not have complex effects on food intake which will complicate the apparently simple result observed under laboratory conditions, and described here. Other factors such as tank size may also have effects on general activity, hence metabolic levels.

Perhaps the most important result emerging from such studies, however, is the suggestion that through use of the balanced energy equation, the factors underlying metabolism are amenable to study in terms which are directly relevant to an understanding of biological production processes. That is, given the recognition of an underlying stable mechanism of regulation, reflected by a predictable relationship between metabolism and body size, possibly even in the simple form of a stable γ as suggested here, we are in a position to carry out direct studies of the influence of environmental factors, such as food supply and fish conditions on general metabolic level, and to apply the results to the interpretation of measurements of these metabolic parameters in nature. The opportunity afforded for understanding the influence of natural conditions on production would justify the considerable work necessary to permit field measurements of metabolism.

It must be noted, however, that information on metabolic rate alone is insufficient as a basis for specifying the production process. In addition to information on energy dissipation in relation to supply, we have also to consider the remainder of the energy system consisting of the component of deposition of energy in the form of growth. Both components may be expected to have important relations to the supply. However, the demonstration here of the apparently simple and stable nature of the mechanisms regulating the relation of dissipation in terms of metabolism to the rate of supply in terms of rations leads easily to the inference that there must be a similarly definable relation of these two components with the growth. The evidence for this, and the relationships apparently involved, are discussed in another paper in preparation (Paloheimo and Dickie, 1966).

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Mechanism of Gaseous Exophthalmia in the Atlantic Cod, *Gadus morhua* L.[1]

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ABSTRACT

Gadus morhua L. in captivity often develop unilateral or bilateral exophthalmia, characterized by the accumulation of gas bubbles in the eye.

Rete mirabile-like organization of capillaries in the choroid "gland" in association with the glandular pseudobranch, which is a rich source of carbonic anhydrase in fishes, are involved in gas secretion in the eye of cod. Intraperitoneal injections of Diamox and pseudobranchectomy prevented exopthalmia in the fish.

Presence of glycogen and carbonic anhydrase in the choroid gland in relation to gas secretion in the eye suggests a parallel with the process of gas secretion in the teleostean swimbladder.

Gas secretion in the eye of cod resulting in exopthalmia would seem to be a malfunctioning of the choroid gland-pseudobranch complex in the fish triggered by unknown stimuli.

INTRODUCTION

Atlantic cod, *Gadus morhua* L., held in captivity often develop unilateral or bilateral exophthalmia (pop-eye) which makes such fish useless for many experimental purposes.

Exophthalmia in cod is characterized by the accumulation of gas bubbles in the eyeball which ultimately lodge in the anterior chamber. This suggests a gas-secreting mechanism within the eye of the fish. Wittenberg and Wittenberg (1962) observed a high tension of oxygen in the retina of Atlantic cod but do not mention the occurrence of exophthalmia in the fish. Secretion of gases is known to occur only in the swimbladder of teleosts. The structure and function of the rete mirabile and gas gland in the teleostean swimbladder in relation to secretion of gases have been reviewed by Jones and Marshall (1953). Fänge (1953) has explained the role of carbonic anhydrase in the process of secretion of carbon dioxide and oxygen which occurs in the physoclistous swimbladder as a result of glycolysis.

The rete mirabile-like organization of the blood capillaries in the choroid gland (Brett, 1957), and its intimate anatomical association with the pseudobranch, which is a rich source of carbonic anhydrase in fishes (Copeland, 1952a; Parry and Holliday, 1960), could be of significance in relation to gas secretion in the eye of cod. The present paper deals with experimental evidence in support of this proposition.

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MATERIALS AND METHODS

Specimens of *Gadus morhua* were captured near St. Andrews, N.B., by otter trawls and were held in tanks with flowing water on the deck of the research vessels. Fish ranging from 150 to 350 g were selected and stocked in large, circular, fibreglass tanks in the laboratory with continuous flow of sea water at 5–6 C. A mixture of liver, herring, and yeast was given to fish as food daily. No supersaturation of the water with air occurred during the experiments.

To compare structural details of normal and exophthalmic eyes, fresh and formalin-fixed specimens of cod were dissected. For histological study the choroid gland attached to the retina and the glandular pseudobranch from freshly killed cod were fixed in aqueous Bouin's fluid. Sections cut at 6–8 μ were stained with Ehrlich's haematoxylin and counter-stained with alcoholic eosin.

To determine glycogen in cells of the choroid gland the tissue from freshly killed cod was fixed in Bouin's picro–formol–acetic acid solution. Sections cut at 6–8 μ were subjected to Hotchkiss' periodic acid–Schiff (PAS) technique (Pearse, 1960). Sections treated with "saliva test" served as controls.

To estimate carbonic anhydrase in the choroid gland, frozen tissue was cut at 45 μ on the cold microtome. The fresh sections were treated with Hausler's (Pearse, 1960) technique. For control, sections were incubated with 0.1% Diamox (Parenteral, Lederle).

Diamox was used as a carbonic anhydrase inhibitor to determine the role of the enzyme. Experimental fish were injected intraperitoneally with Diamox dissolved in distilled water. A dose of 0.5 mg/100 g body weight of the fish was used. Control fish were injected intraperitoneally with equivalent quantities of saline water.

Bilateral pseudobranchectomy was conducted to assess the possible role of the pseudobranch in the gas-secreting mechanism in the eye of cod. Pseudobranchectomy was performed on unaesthetized cod restrained under wet cloth. When the operculum is lifted, the pseudobranch is clearly visible lying superficially on the anterodorsal side under the preopercular region. The pseudobranch was removed by cutting around it with a hooked scalpel and then lifting the organ off with small forceps. The operation caused little bleeding. In sham operations on control specimens scalpel incisions were made near the pseudobranch.

To study the effect of Diamox injections and pseudobranchectomy, the fish were subjected to experiments immediately after they were brought to the laboratory.

Statistical significance of the observations on the effects of Diamox injections and pseudobranchectomy were tested by Fisher's exact probability test.

OBSERVATIONS

Nature of Exophthalmia

When cod were kept in large, fibreglass tanks 17 of 24 fish developed exophthalmia (Fig. 1) within 7 days after capture. Exophthalmia was either unilateral or bilateral. The exophthalmic condition was observed to be a symptom of the accumulation of gases within the eyeball. Gas bubbles gradually increased and ultimately lodged in the anterior chamber causing considerable bulging of the eye and often resulting in haemorrhage. Rate of increase in size of bulge varied in different individuals.

Exophthalmic cod kept for a prolonged period in the tank were often seen floating with the abdomens up.

Structure of Choroid Gland and Glandular Pseudobranch

The large choroid gland is situated between the retina and the sclera of the eye and forms a layer investing almost the entire retina. The outer surface is connected with the sclera by loose connective tissue. Its inner surface is loosely attached to the retina. The choroid gland is formed of myriads of fine blood vessels in the form of retia mirabilia. Interspersed between the blood vessels is the delicate cellular stroma on the inner side of the choroid near the retina (Fig. 2).

The choroid gland in cod receives its blood supply directly from the pseudobranch as in other teleosts. The arterial supply to the pseudobranch forms a capillary system within the organ and after reuniting to a single efferent ophthalmic vessel forms a second capillary system in the choroid gland of the eye. Histologically, the structure of the pseudobranch of cod conforms to that described in other fishes (Copeland, 1952a).

The choroid gland in the exophthalmic eye of cod showed hypertrophy and formed a spongy mass of dark red tissue around the retina. The blood vessels were dilated as observed histologically.

Glycogen and Carbonic Anhydrase in Choroid Gland

PAS reaction showed the presence of glycogen in the cellular part of the choroid gland. Concentration of glycogen appeared to be confined mostly towards the inner border of the choroid gland tissue. Staining for glycogen in the cells of the choroid gland from the normal eye was dense (Fig. 3) but that in the choroid gland tissue from the eye which had shown the onset of exophthalmic condition was faint (Fig. 2).

Presence of carbonic anhydrase was profuse in the choroid gland tissue of the eye with developing exophthalmia (Fig. 4) while in the choroid gland of the normal eye of cod the activity of the enzyme was comparatively meagre (Fig. 5), as indicated by scanty deposition of cobalt sulphide.

Effect of Intraperitoneal Injections of Diamox

Two groups of 10 fish each were selected and were kept in separate fibreglass tanks of 61 × 36 × 20 cm in size with a flow of running sea water at 5–6 C.

Experimental fish were injected intraperitoneally with 0.5 mg/100 g of body weight every 24 hr for the first 3 days. Later the dosage was injected on alternate days until the seventh day of the experiment, when injections were discontinued. Control fish were injected intraperitoneally with equivalent quantities of saline water. Observations were continued for 21 days.

For the first 4 days the experimental fish were lethargic, became dark in colour, and stopped feeding. Two days after the Diamox injections were discontinued, fish resumed normal activity and feeding and regained the normal colour. There was one death on the second day. None of the fish injected with Diamox showed exophthalmia during the period.

In the control group, three fish showed unilateral exophthalmia on the second day and by the seventh day five fish were exophthalmic. Out of these one showed bilateral exophthalmia.

The results were significantly different at the 0.05 level.

Effect of Pseudobranchectomy

A group of 10 fish after bilateral pseudobranchectomy was kept in a fibreglass tank 61 × 36 × 20 cm in size. A control group of 10 fish was kept in a similar tank. A flow of running sea water at 5–6 C was maintained in the tanks. The fish were offered food regularly but few responded. There was one death in the control group on the sixth day.

None of the fish in which the pseudobranch was removed showed exophthalmia. In the control group of 10 fish, six fish showed noticeable exophthalmia within 4 days, of which four had unilateral and two had bilateral exophthalmia.

These results were significantly different ($p > 0.05$).

DISCUSSION

Various causes of exophthalmia in trout and other species of fishes are known (Davis, 1953). A pop-eyed condition due to the accumulation of nitrogen in the eye may occur in fishes when their water supply becomes supersaturated with air (March and Gorham, 1905). Such a cause in the present experiment with cod can be ruled out since no supersaturation occurred in the water supply. Moreover, the effects of ultraviolet light and physical damage in the laboratory have been investigated to find the possible cause of the pop-eyed condition, but without success (Anon., 1963). In the present study, evidence has been presented that exophthalmia in cod is caused by the active secretion of gases within the eye.

The analyses of gases ($CO_2 = 0–0.7\%$, and $O_2 = 10–35\%$) from the exophthalmic eye of cod (J. Demelker, unpublished data) show the contents

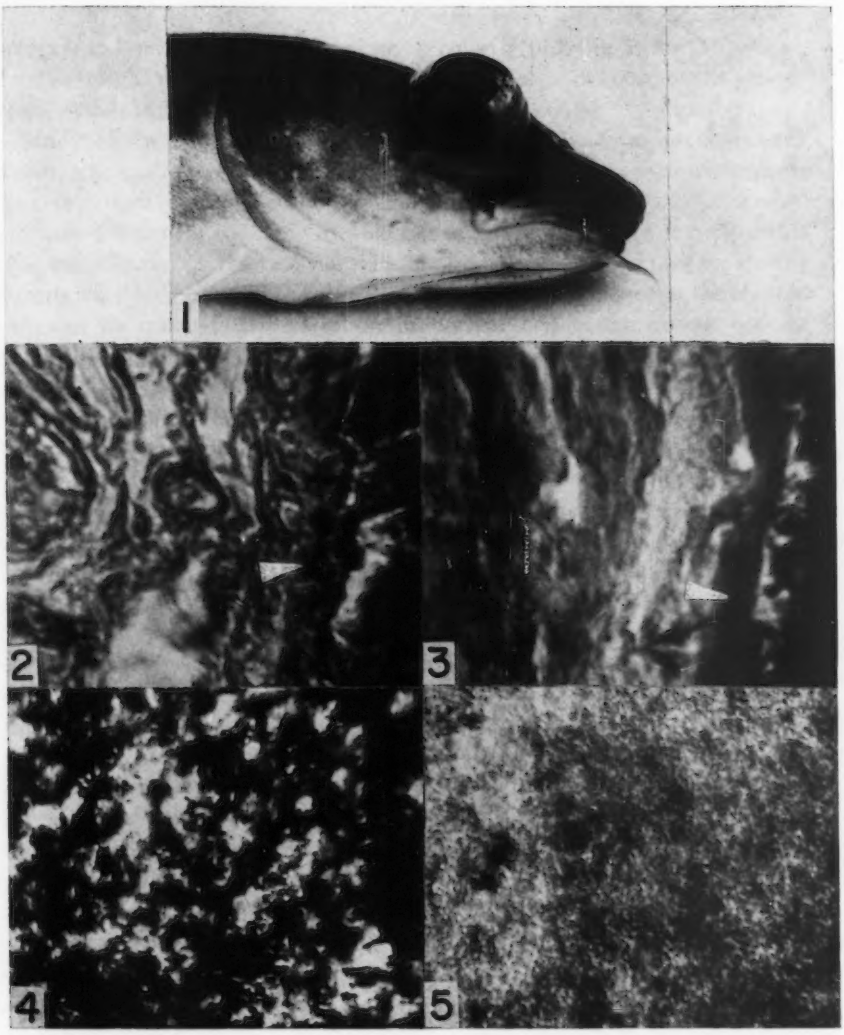

FIG. 1. Exophthalmia in *Gadus morhua* due to accumulation of gases in the eye.

FIG. 2. Transverse section through choroid gland and retina of developing exophthalmic eye of cod showing traces of glycogen in choroid gland (8 μ; 44 $\times$ 15; PAS).

FIG. 3. Transverse section through choroid gland and retina of normal eye of cod showing dense concentration of glycogen (8 μ; 44 $\times$ 15; PAS).

FIG. 4. Transverse section through choroid gland in developing exophthalmic eye of cod showing intense activity of carbonic anhydrase as indicated by the heavy deposition of cobalt sulphide (45 μ; 15 $\times$ 10).

FIG. 5. Transverse section through the choroid gland in normal eye of cod showing meagre activity of carbonic anhydrase as indicated by scanty deposition of cobalt sulphide (45 μ; 15 $\times$ 10).

of oxygen and carbon dioxide sometimes greater than those in air. Moreover, the values of gas constitution appear to be in the range of those in the physoclistous swimbladder of teleosts (Saunders, 1953).

Study of the choroid gland and the associated glandular pseudobranch in relation to gas secretion in the eye of cod shows a parallel with the process of gas secretion in the teleostean swimbladder. Enlargement of the choroid gland and dilation of its capillaries in the exophthalmic eye are comparable to the vasodilation in the rete mirabile of the teleostean swimbladder during gas secretion (Hall, 1924; Fänge, 1953). The presence of glycogen in the choroid gland and its quantitative variation in normal and exophthalmic eyes of cod may be significant in relation to gas secretion. Copeland (1952b) and Fänge (1953) point out the important role of glycogen during gas secretion in the swimbladder. Finally, greater activity of carbonic anhydrase in the choroid gland of exophthalmic eyes in comparison with normal eyes of cod suggests a role of this enzyme in the gas-secreting process. The result of intraperitoneal injections of Diamox which inhibited the process adds further evidence to the role of carbonic anhydrase. Carbonic anhydrase is known to play an important part in the mechanism of gas secretion in the physoclistous swimbladder (Copeland, 1952a; Fänge, 1953).

Effects of pseudobranchectomy which blocked the gas secretion in the eye of cod indicates the direct role of the pseudobranch probably as a source of carbonic anhydrase as suggested by Copeland (1952a) and Parry and Holliday (1960).

Evidence in the literature is recorded that the choroid gland and pseudobranch function as a single unit (Parry and Holliday, 1960). The present observations indicate that the choroid gland and the glandular pseudobranch are involved in the secretion of gases which cause exophthalmia in cod. However, the formation of gas bubbles in the eye of cod would seem to be a malfunctioning of the choroid gland-pseudobranch complex triggered by unknown stimuli. Further research on the factors which cause pop-eyed condition in cod is warranted.

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NOTES

Vitamins B in Dulse (*Rhodymenia palmata*)

Dulse (*Rhodymenia palmata*) belongs to the group of red algae, Rhodophyceae, and is widely distributed along the eastern coast of Maine, New Brunswick, and Nova Scotia. The production of dry dulse in Nova Scotia was reported as 2000 lb in 1964, and it is the only edible seaweed commercially available in Canada. However, only limited nutritional data are available as follows (O. Hjelkrem, Bonda Foods Ltd., Yarmouth, N.S., personal communication): protein 25.3%, lipid 3.8%, cellulose 1.5%, ash 26.7%, and carbohydrate (soluble) 44.2%. The inorganic components are (in per cent): Na 2.5, K 7.1, Ca 0.47, Mg 0.22, I 0.02, P 0.32, Cl 6.4. Trace elements are (*ppm*): Co 0.47, Ni 2.0, Mo 0.37, Fe 1500, Zn 100, Cu 26, and Mn 10.

The biochemistry and physiology of vitamins in algae have been reviewed by one of the authors (Kanazawa, 1963) but there is no report on Canadian dulse.

The sample of dulse was collected at Grand Manan, New Brunswick, in midsummer of 1964. It was sun-dried and stored in a dark, unheated warehouse for 8 months. Vitamin-B analyses were conducted as soon as the dulse arrived at this laboratory. The results of the vitamin-B group analyses are presented in Table I.

These values were within the average range of available data with the exception of thiamine which was higher. The vitamin-B content of 20 species of red algae grown in Japan was reported as (μg/g dry matter): thiamine 0.04 – 4.60, riboflavin 0.38 – 23.08, nicotinic acid 2.00 – 68.33, panthothenic acid 1.22 – 12.21, biotin 0.018 – 0.294, and vitamin B_{12} 0.015 – 0.290 (Kanazawa, 1962, 1963). The thiamine content of other Rhodophyceae are reported as 2.88 μg/g of dry matter in *Chondrus crispus*, 3.04 in *Rhodomela subfusca*, 2.82 in *Porphyra laciniata* (Gerdes, 1951), 0.83 in *Chondrus crispus* (Terri and Bieber, 1958), 2.19 in *Chondrus ocellatus*, and 4.60 in *Grailaria textorii* (Kanazawa, 1963).

Vitamin analyses have generally been conducted on fresh seaweeds. Drying and storage have no doubt promoted the destruction of vitamins-B in the dulse and sun-drying would have an adverse effect on thiamine and riboflavin. It is of interest that, in spite of adverse circumstances, dulse contained a high level of thiamine and normal levels of the other B vitamins. These data suggest that dulse is a useful food and animal feed supplement.

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(Table I *on next page*)

TABLE I. Vitamin bioassay of dulse (*Rhodymenia palmata*).

Vitamins	Preparation of samples	Test organisms; incubation time; method	Results ($\mu g/g$ *dry matter*)
Thiamine	The sample (4 g) was heated at 100 C with 10 vol of N/10 H_2SO_4 for 30 min, hydrolyzed with 200 mg of Takadiastase (37 C, 24 hr, *p*H 4.5); *p*H adjusted to 6.6	*Lactobacillus fermenti* 36 (ATCC 9338) 37 C, 16 hr; turbidimetry	6.32
Riboflavin	The sample (1 g) was hydrolyzed with N/10 HCl at 15 lb for 15 min, *p*H adjusted to 4.5, filtered, *p*H adjusted to 6.8	*Lactobacillus casei* (ATCC 7469) 37 C, 18 hr; turbidimetry	5.00
Nicotinic acid	The sample (1 g) was hydrolyzed with 10 ml of 1 N H_2SO_4 at 15 lb for 30 min; neutralized to *p*H 7.0	*Lactobacillus arabinosus* 17-5 (ATCC 8104) 37 C, 72 hr; titration	16.91
Vitamin B_6[a]	The sample (1 g) was hydrolyzed with 50 ml of 0.44 N H_2SO_4 at 20 lb for 1 hr; adjusted *p*H to 5.2	*Saccharomyces carlsbergensis* 4228 (ATCC 9080) 30 C, 15–16 hr; turbidimetry	0.143
Panthothenic acid	The sample (1 g) was suspended in 0.2% acetic acid with 0.4 g of Mylase P, at *p*H 4.5; extracted at 50 C for 3 hr; neutralized to *p*H 6.8	*Lactobacillus arabinosus* 17-5 (ATCC 8014) 37 C, 72 hr; titration	4.30
Biotin	The sample (1 g) was hydrolyzed with 25 ml of 2 N H_2SO_4 at 15 lb for 1 hr; neutralized to *p*H 6.8–7.0	*Lactobacillus arabinosus* 17-5 (ATCC 8014) 37 C, 72 hr; titration	0.182
Vitamin B_{12}	The sample (1 g) was hydrolyzed with KCN (1 mg/μg of B_{12}) at *p*H 4.5–5.0 at 15 lb for 15 min; *p*H adjusted to 6.0	*Lactobacillus leichmannii* (ATCC 4797) 37 C, 18–24 hr; turbidimetry	0.028

[a]Pyridoxal + pyridoxamine + pyridoxine.

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Evidence for the Binding of Oil in Salmon Canned After Storage in Ice or in a Partially Frozen Condition

In the course of an investigation of the usefulness of partial freezing as a means of preserving Pacific salmon intended for canning (Tomlinson et al., 1965) it was observed that storage of the fish in either refrigerated sea water (RSW) at 30.5 F, or in refrigerated, salt-fortified sea water (RFSW) at 25–28.8 F, occasioned an apparent decrease in the quantity of free oil in the subsequently canned product. That frozen Pacific salmon yield a canned product with a lower free oil content than do fish not previously frozen has been recognized for some time (Stansby and Dassow, 1951), but there appears to be no known explanation for the phenomenon. A similar difference in free oil between alewife (*Pomolobus pseudoharengus*) canned fresh and others canned after a period of frozen storage has been observed (C. J. Carlson, Bureau of Commercial Fisheries, Gloucester, Mass., personal communication), and recently an apparent large decrease in the oil content of whiting (*Merluccius bilinearis*) during storage in RSW and in ice has been reported by Cohen and Peters (1963), although these authors indicated the decrease was probably fortuitous.

The matter has now been investigated further, with the result that the apparent decrease in free oil that occurred in Pacific salmon canned after storage in RFSW has been shown to be real. It has also been shown that there is no corresponding decrease in the total lipid content of the canned product. However, it has been found that a decrease in free oil of about the same magnitude occurs in iced fish, but more slowly. This does not seem to have been recognized previously. Details are given below.

Sockeye salmon (*Oncorhynchus nerka*) were obtained within 24 hr of capture. Storage and canning procedures have been described previously (Tomlinson et al., 1965). Each can was marked in such a way that it was possible to identify not only the fish from which its contents came, but also the section of the carcass. This was done in order that reliable comparisons could be made between treatments, since different parts of the carcass may have different oil contents.

For analysis, cans prepared from the section of the carcass beneath the dorsal fin were selected. A can and its contents were weighed, the can was opened, its contents turned out onto a large funnel, and allowed to drain for 7 min, the oil and water mixture being collected in a graduated centrifuge tube. The tube and its contents were then centrifuged and the volume of the oil layer was measured. The weight of the empty can and its lid were measured and the net weight of its contents determined and recorded. The liquid and solid portions of the can contents were recombined and homogenized in a Waring blendor until a homogeneous suspension was obtained, after which

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TABLE I. Influence of previous storage in ice or in RFSW at 25 F on the free oil and total lipid content of canned sockeye salmon.

Period of storage (*days*)	Method of storage	Number of fish	Free oil (*ml/100 g canned fish*)	Total lipid (*g/100 g canned fish*)	Free oil (*ml/100 g total lipid*)
1	Ice	6	2.33±0.33 (16)[a]	14.08±0.80 (16)	15.0±1.58 (16)
3	Ice	6	2.43±0.32 (16)	15.64±1.16 (16)	14.5±1.49 (16)
10	Ice	6	1.04±0.09 (18)	13.84±0. 52(18)	7.5±0.57 (18)
3	RFSW 25 F	6	1.22±0.09 (18)	14.96±0.48 (18)	8.1±0.77 (18)
10	RFSW 25 F	6	1.34±0.13 (18)	15.72±0.56 (18)	7.9±0.55 (18)

[a]Average values are given together with the standard error followed by the number of determinations in parentheses.

TABLE II. Statistical comparison of differences between treatments.

Treatments compared	Probability (P^a) of the significance of differences between the quantities of free oil in canned fish	total lipid
1 day iced 3 days iced	>0.5	>0.5
1 day iced 10 days iced	<0.01*	<0.01*
3 days iced 10 days iced	<0.01*	<0.01*
1 day iced 3 days RFSW 25 F	<0.01*	<0.01*
1 day iced 10 days RFSW 25 F	<0.01*	<0.01*
3 days RFSW 25 F 10 days RFSW 25 F	0.5	>0.5
3 days iced 3 days RFSW 25 F	<0.01*	<0.01*
10 days iced 10 days RFSW 25 F	0.1	>0.5

[a]Calculated by application of "*t*" test to the data from Table I. An asterisk indicates the probability level at which the differences between the averages are highly significant ($p \leq 0.01$)

the total lipid content was determined on a suitable aliquot by the method of Bligh and Dyer (1959).

The results are recorded in Tables I and II. It will be seen that after both 3 and 10 days of storage in RFSW, and after 10 but not after 3 days in ice, there was a highly significant decrease in the millilitres of free oil found per 100 g of canned fish and also in the millilitres of free oil per 100 g of total lipid in the canned fish. The decrease in free oil occurred more quickly in fish stored in RFSW than in those stored in ice, but by the tenth day of storage there was no significant difference between fish stored by the two methods. The fact that there was no loss in total lipid clearly suggests that some change had occurred during storage that resulted in a binding of oil. However, whether the binding occurred during storage, or during canning as a result of some preceding change during storage, and the nature of the binding itself, are at present unknown.

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Nucleotide Interference with Glycogen Estimation in White and Red Muscle of Cod

The estimation of glycogen in muscle tissue still presents problems (see Anon., 1965). The usual acid procedures give incomplete extraction, leaving "residual glycogen" in the residue, when compared with the classical Pflüger alkaline (KOH) digestion for total glycogen, but recently, Roe et al. (1961) have shown that glycogen can be extracted completely by acid under drastic and specialized conditions of homogenization. Residual glycogen can also be extracted by KOH digestion, and, together with the acid-extractable fraction, yields total glycogen equal to that obtained by direct KOH digestion of the tissue (Russell and Bloom, 1955).

When nucleotides and other glycolytic metabolites are being extracted with perchloric acid, it may be convenient to determine total glycogen as the sum of that in a $HClO_4$ extract and in a KOH digest of the residue. On investigating this, we frequently observed that more glycogen was precipitable by alcohol from $HClO_4$ extracts neutralized to minimize breakdown of acid-labile metabolites than from the same extracts left un-neutralized. The presence of nonglycogen carbohydrate was investigated by comparison of results obtained by using a general carbohydrate reagent (phenol-sulphuric acid (PS)) and by an enzymic procedure (glucose oxidase) specific for glucose.

The results of a typical experiment on stunned unexercised cod sampled within 5 min of death (Table I) showed that nonglycogen carbohydrate was not precipitated with alcohol from un-neutralized $HClO_4$ or from KOH extracts, since PS and Glucostat determinations were in substantial agreement. In the neutralized $HClO_4$ extracts, however, approximately 24–27% of PS-reactive carbohydrate was precipitable by alcohol in addition to the glucose-yielding material determined by Glucostat. This alcohol-precipitable material redissolved in water absorbed strongly in the ultraviolet, with maxima and minima at 259 and 227.5 mμ, respectively, indicating adenine nucleotide compounds. Paper chromatography in the isobutyric acid system of Krebs and Hems (1953) produced three spots running in the positions of markers ATP, ADP, and AMP. The percentages of total adenosine and inosine compounds (TN) co-precipitated with glycogen by alcohol from the neutralized $HClO_4$ extracts of white and red muscle were 40 and 56% of those originally present, respectively (Table II), while from both, 70% of the adenine nucleotide was precipitated with alcohol. The presence of adenine nucleotide was further substantiated by determination of ribose and acid-labile phosphorus in the redissolved precipitate in amounts which agreed with the nucleotide found. A much smaller proportion (about 15%) of the inosine compounds was precipitable by alcohol. The co-precipitated nucleotide material could be removed from solution by shaking with Dowex 1 (Cl^-) (Jones and Murray, 1964);

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TABLE I. Glucose equivalents (mg/100 g wet muscle) in white and red muscle of cod, as determined by Glucostat and by phenol-sulphuric acid, on the alcohol-precipitable fraction and directly on extracts (total reactives) from $HClO_4$ extraction or KOH digestion. Duplicate precipitations and determinations were performed throughout.[a]

Extract	White muscle		Red muscle	
	Phenol-sulphuric	Glucostat	Phenol-sulphuric	Glucostat
$HClO_4$ — un-neutralized				
Alcohol-precipitable reactives	254, 245, 254 / 243, 244, 252 } 249	262 / 268 } 265	371 / 360 } 366	418 / 392 } 405
Total reactives	562 / 557 } 560	311 / 305 } 308	530 / 598 } 564	428 / 413 } 420
Free Glucose		18		30
$HClO_4$ — neutralized				
Alcohol-precipitable reactives	315 / 299 } 307	230 / 238 } 234	413 / 409 } 411	290 / 307 } 299[b]
Total reactives	527 / 563 } 545	279 / 268 } 273	593 / 554 } 574	355 / 360 } 358
Free glucose		20		29
KOH digest of $HClO_4$ residue				
Alcohol-precipitable reactives	53 / 59 } 56	61 / 67 } 64	60 / 67 } 63	23 / 25 } 24
Total reactives	57 / 73 } 65	–	67 / 95 } 81	–
KOH digest of muscle				
Alcohol-precipitable reactives	317 / 326 } 321	325 / 333 } 329	492 / 495 } 494	472 / 518 } 495
Total reactives	537 / 579 } 558	–	518 / 570 } 544	–

[a]One of two adjacent muscle samples was digested with 30% KOH (Good et al., 1933) at 100 C for 1 hr; the other was homogenized (Lourdes Multi-mixer) with 4–6 vol of ice-cold 3% $HClO_4$ for 3–5 min followed by centrifugation and washing of the residue. Filtration must be avoided since filter paper particles are PS-reactive. A portion of the combined supernatants was neutralized to *p*H 6.5–7.2 with 30% KOH. Residual glycogen was extracted from the residue with hot KOH as above. Glycogen was precipitated from the $HClO_4$ extracts with 5 vol of 95% ethanol (Carroll et al., 1956), and from the KOH digests with 1.2 vol of alcohol (Good et al., 1933), and determined in the redissolved precipitates both by phenol-sulphuric acid (Montgomery, 1957), and by glucose oxidase reagent (Glucostat Special, Worthington Biochemical Corporation, Freehold, N.J.) after hydrolysis for 2 hr in 2 N H_2SO_4 at 100 C and neutralization to *p*H 7.0. The yellow color of the KOH digests interfered with the estimation of total reactives by the Glucostat procedure.

[b]This value is unaccountably low; in other experiments such a discrepancy was not encountered.

after this treatment the glycogen content as determined by PS reagent closely approximated that measured by Glucostat prior to and following Dowex shaking. It was also found that shaking of the neutralized $HClO_4$ extracts with Dowex prior to alcohol precipitation removed the interfering nucleotide material, so that subsequent determination of glycogen in the redissolved alcohol precipitate by the PS reagent and by Glucostat yielded results in close agreement. The problem of nucleotide material co-precipitating with glycogen was encountered only with the neutralized $HClO_4$ extracts; nucleotide was not detected in the material precipitated with alcohol from un-neutralized $HClO_4$ extracts or from KOH digests.

The presence of free glucose and possibly glucose-1-PO_4 in the $HClO_4$ extracts of both red and white muscle is indicated since the total Glucostat-reactive glucose (following hydrolysis) in the extract was 4–16% higher than in the alcohol precipitable "glycogen" (Table I). Free glucose in the extracts (determined by Glucostat without hydrolysis) accounted for roughly half of

TABLE II. Adenosine compounds (AN) and total adenosine and inosine compounds (TN) in the neutralized $HClO_4$ extracts of red and white muscle and in the alcohol-precipitable fraction.

	TN[a]	AN	Per cent AN
White muscle	*μmole/ g*	*μmole/ g*	
Neutralized $HClO_4$ extract	8.5	4.0	47
Alcohol-precipitable fraction	3.4	2.8	82
Red muscle			
Neutralized $HClO_4$ extract	7.7	5.8	76
Alcohol-precipitable fraction	4.3	4.0	93

[a]Calculated from the absorption at 251 mμ, the wavelength of the isobestic point for purified samples of Schwartz AMP and IMP (J. R. Dingle, personal communication), and AN from the absorption at 260 mμ.

the differences found. Burt (1961) found 40 and 17 mg/100 g of free glucose and glucose of glucose-1-PO_4, respectively, in rested cod muscle; the former may reach levels of 60 mg/100 g during postmortem storage while glucose-1-PO_4 decreases. Glucose-6-PO_4 is relatively stable to acid hydrolysis (3% hydrolyzed after 2 hr in 2 N H_2SO_4 at 100 C) and therefore would not interfere at the concentrations found by Burt (1961). It appears, therefore, that a measure of the glycogen content of acid extracts might be given by the difference between free glucose and total glucose (after hydrolysis) without the necessity of the alcohol precipitation step, provided that glucose-1-PO_4 does not make an appreciable contribution.

The much higher values for "total reactives" in the extracts as measured with the PS reagent compared with those of total hydrolyzable glucose (Glucostat reagent) are undoubtedly due to free glucose and sugars contributed to varying extents by nucleotides and the hexose phosphates of the glycolytic

cycle. The total carbohydrate content was the same in the un-neutralized and neutralized extracts of both white and red muscle, indicating that no carbohydrate material had been brought down along with $KClO_4$ during the neutralization.

A total glycogen value 87–96% of that obtained by direct KOH digestion was given by the sum of the acid-extractable and residual glycogens (Table I). Similar values are reported for various rat tissues (Russell and Bloom, 1955). With cod muscle of low glycogen content, 20–100 mg/100 g, and in frozen muscle, we often observed that the proportion extractable by $HClO_4$ was low (about 50% of that obtained by alkali digestion), but the sum of the extractable and residual glycogens agreed with that given by direct KOH digestion. This low acid extractability is comparable with that found as a result of fasting, muscle work, and epinephrine and insulin injection (see Stetten and Stetten, 1960; Roe et al., 1961).

On the basis of the present findings, we suggest that total glycogen in unfrozen fish muscle may be extracted either by boiling KOH or, if convenient, by cold $HClO_4$ followed by KOH digestion of the residue, and the alcohol-precipitable glycogen determined either with the PS reagent (or anthrone) directly, or by Glucostat after acid hydrolysis. With tissues of low glycogen content (less than 50 mg/100 g), however, the Glucostat procedure should be used initially in conjunction with the PS reagent to check the possibility of interference with the latter from significant amounts of nonglycogen carbohydrate. Un-neutralized $HClO_4$ extracts should be used for the alcohol precipitation of glycogen, thus eliminating the co-precipitation of nucleotides.

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Influence of Fishing Methods on the Incidence of Chalkiness in Halibut

During a recent investigation into the cause of chalkiness in halibut (*Hippoglossus stenolepis*) (Tomlinson et al., 1965), the incidence of chalkiness encountered in fish taken by dragging (bottom trawling) was found to be higher than that usually met with in fish taken by longline. Two possible explanations for the difference, each based on the finding that development of chalkiness occurs when flesh *p*H falls postmortem to about 6.0 or lower, were suggested. The first was that dragging would take heavily fed fish that might not be taken by longline — heavily fed fish, in consequence of their lower flesh *p*H postmortem, being more likely to become chalky than those less well fed. The second was that the difference in time between the catching and killing of fish caught by the two methods, as usually practiced, would result in longlined fish being allowed a more or less lengthy period of recovery on the hook before death, whereas no such recovery period would be enjoyed by the trawl-caught fish. It might then be that the fish allowed the period of recovery (longlined) would not attain as low a flesh *p*H postmortem as would the fish killed without a recovery period (dragged) and consequently would be less likely to become chalky. Evidence in support of the second possibility has been provided by Black et al. (1962), who found that in rainbow trout (*Salmo gairdneri*) recovering from exercise, the lactic acid content of muscle was greatly reduced after a few hours recovery while only a portion of the muscle glycogen present before exercise had been reformed. It seems likely that a fish killed under such conditions would have a lower concentration of lactic acid in its flesh postmortem and hence a higher flesh *p*H than would a fish killed in either a completely unexercised or in a freshly exhausted state.

To examine the validity of the second of the suggested explanations, three experiments have now been carried out. In each of these, halibut were caught by dragging, then, as the fish were removed from the net, they were subjected alternately to one of two different procedures. In the first treatment, the fish was killed at once, eviscerated and iced. In the second, a halibut hook attached to a short length of line was inserted in the mouth of the fish after which the fish was placed in a tank through which fresh sea water (temperature 9–10 C) was pumped continuously. Fish subjected to the latter treatment were then allowed to remain in the tank for various periods of time in order to simulate conditions they would meet if caught by longline, after which time they were removed from the tank, killed, eviscerated, and iced. The procedure of alternate selection of fish from the net was used in order to randomize sampling. Previous experience has shown that wide variations in incidence of chalkiness could occur in fish taken in different drags (Tomlinson et al., 1965) and that the size of fish was a possible source of

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influence on the development of chalkiness (Patashnik, 1966; Tomlinson et al., 1966). The fish were transported to the laboratory and, at the end of 11 days' storage in ice, the *p*H of their flesh was measured and they were examined for development of chalkiness. Measurement of flesh *p*H was made by means of a combinations glass electrode inserted directly into a small incision, about 1 inch deep, cut midway in the length of the fish and 2–3 inches from the edge of the body away from the "poke" or body cavity. Degree of chalkiness was judged by visual examination of a cut made across the widest portion of the body. Fish were classified as chalky when the flesh was dull, white and opaque; borderline, when shiny, white and nearly opaque; nonchalky, when shiny and translucent.

The results of two of the three experiments are recorded in Table I. In each of these experiments, the mean flesh *p*H was higher, and the incidence of chalkiness lower, in the group allowed the period of recovery than in the corresponding group killed at once. However, a lower overall mean flesh *p*H (6.06) was met with in experiment 1 than in experiment 2 (6.35). In consequence, as would be expected from what is known of the relation between flesh *p*H and chalkiness (Tomlinson et al., 1965, 1966; Patashnik, 1966), and since in each experiment the increase in mean flesh *p*H resulting from recovery

TABLE I. The influence of a recovery period between the catching and killing of halibut on flesh *p*H postmortem and on incidence of chalkiness.

	Fish killed	
	Immediately after being caught	After a period of recovery[a]
Experiment 1		
Number of fish	13	13
Mean flesh *p*H[b]	5.98	6.14
Range of flesh *p*H	5.74–6.45	5.78–6.66
Percentage of fish chalky of borderline	77	46
Experiment 2		
Number of fish	21	21
Mean flesh *p*H[b]	6.23	6.47
Range of flesh *p*H	5.90–6.64	6.25–6.85
Percentage of fish chalky or borderline	33	0

[a]Recovery period: experiment 1, 10 hr; experiment 2, 9–13 hr. A struggle lasting about 30 sec occurred during the killing of each of these fish.

[b]Analysis of variance applied to the data of experiment 1 indicates that the difference in *p*H observed is significant at $P = 0.2$ but not at $P = 0.1$. For experiment 2 the difference in *p*H observed is significant at $P = 0.01$.

was of roughly the same magnitude, the percentage reduction in incidence of chalkiness was smaller in experiment 1. The third experiment gave results very similar to those of experiment 2, the difference in *p*H between the two treatment groups again being significant at P = 0.01. When the data from all three experiments were combined and treated as if from a single experiment the difference in *p*H between the two different treatment groups was also significant at P = 0.01.

The evidence presented supports the view that the time that elapses in longlining between the catching and killing of fish exerts an influence that can in fact result in a higher postmortem flesh *p*H and consequently a lower incidence of chalkiness than would be observed in the same fish caught by dragging. It also indicates that the effectiveness of the time lapse (recovery period) in reducing the incidence of chalkiness will tend to vary from catch to catch with the mean postmortem flesh *p*H of each particular catch. The lower the mean *p*H, the greater will be the proportion of fish whose potential postmortem flesh *p*H is too low to be raised sufficiently during the recovery period to prevent development of chalkiness, i.e. to about *p*H 6.1 or higher (Tomlinson et al., 1966). The reasons for the observed differences between catches in mean *p*H and incidence of chalkiness are not known, but it seems probable that the state of nutrition of the fish and its physical activities in the hours preceding capture would be among them. The possibility that dragging will take heavily fed fish that would not be taken by longlining remains, and may well eventually also be found to have an important influence in determining the difference in incidence of chalkiness in halibut caught by the two methods.

The finding that the postmortem *p*H of halibut muscle can be raised by a simple antemortem treatment of the fish, and that this increase in *p*H is accompanied by a reduction in incidence of chalkiness, provides further support for the view that chalkiness is indeed caused by low flesh *p*H and not by some unknown pathological or abnormal condition (Bailey, 1950; Tomlinson et al., 1965).

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Micro Starch Gel Electrophoresis

Several micro-adaptations of the starch gel technique of Smithies (1955) are in current use in various laboratories. For example, Daams (1963) carried out mouse serum protein analyses on microscope cover slips, using starch gel of 1 mm thickness. Ramsey (1963) separated esterases from the duodenal contents of young calves by thin-layer (about 1.5 mm) starch gel on glass slides. Helleman and Gartner (1964) detected fast-moving hemoglobin fractions using gel poured into moulds of 2.5 mm in depth. Koch et al. (1964) were able to demonstrate ontogenetic changes in the hemoglobins of *Salmo salar* by an ingenious micro modification of the technique on microscope slides.

Considerable changes in multiple hemoglobin patterns of fishes are introduced upon the storage of red cells of their hemolysed solutions (Tsuyuki et al., 1965). Many of these disadvantages can be avoided by field analysis of the hemoglobins. At stationary locations, most of the conventional methods as well as the micro modifications already in use in the various laboratories can be applied. In fisheries research, however, many of the species of interest are marine forms and some time is required to transport the blood samples to convenient land-based laboratories. We have developed, therefore, a micro modification of the starch gel technique which can be used conveniently both at stationary locations as well as on research vessels on the high seas where most known techniques do not allow for motion.

The unit is constructed of plexiglass (Fig. 1) and consists basically of two rows of eight sealed compartments each connected together by lengths of platinum wires ending in suitable terminals for connection to a power supply. The 16 sealed compartments are filled with bridge buffer solutions consisting of 0.3 M sodium borate, *p*H 8.5 (Tsuyuki et al., 1965) to a height of about $\frac{1}{2}$ inch below the open top. This arrangement allows for the simultaneous analyses of 40 samples without spillage of the buffer solutions under constant motion of the vessel. The entire unit with the gels in place can be run in a refrigerator. If no refrigerator space is available, as is frequently the case, 2-dr polyethylene-capped vials could be filled with water, deep-frozen and layered in the trough beneath a $\frac{1}{16}$ inch plexiglass sheet which fits on supports recessed $\frac{1}{16}$ inch below the top surface of the compartments. This plexiglass plate serves as support for the gel slices.

The dimensions of the unit in inches are shown in their top and end views in Fig. 2. The end view also shows the bevelled design of the bottom section of the buffer compartment. A square design at this juncture will cause gas bubbles formed during electrophoresis to collect, causing displacement of the buffer solutions in the compartments and leading to variations in potential differences in the gels.

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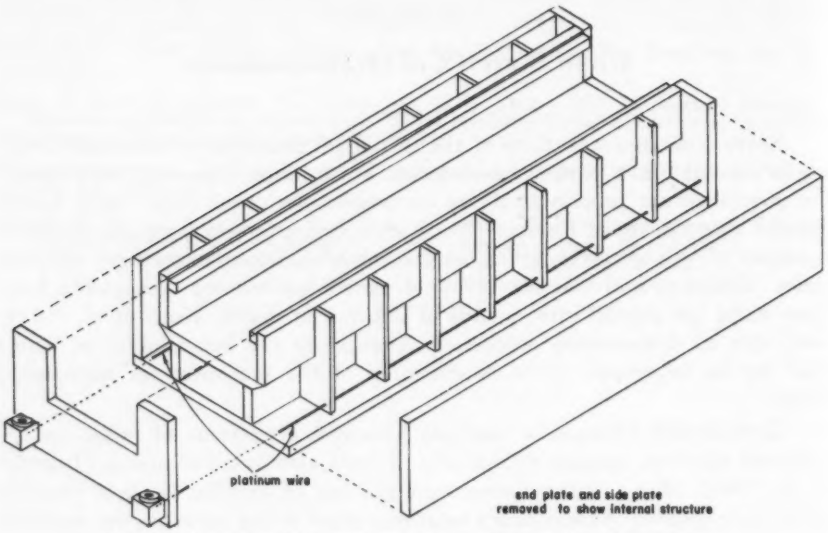

FIG. 1. Overall and internal construction of electrophoresis apparatus.

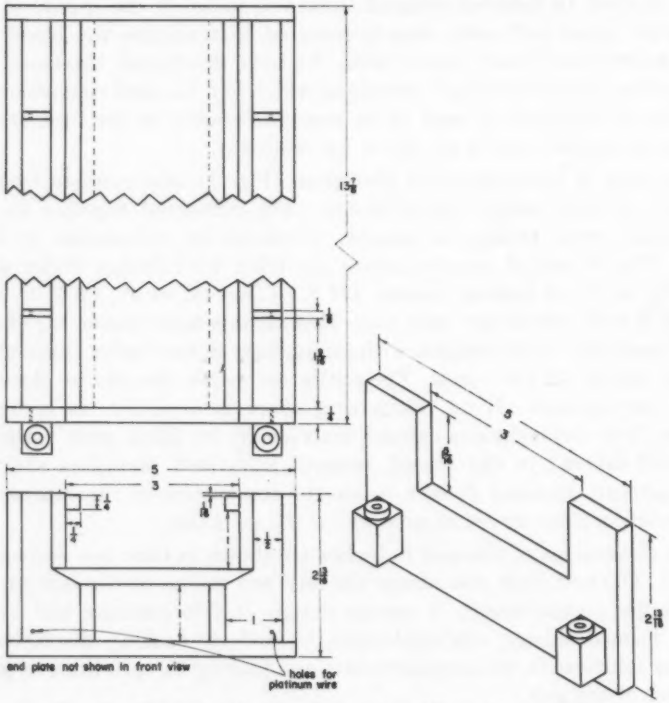

FIG. 2. Top and end views of apparatus (dimensions in inches).

The starch gel is prepared in the usual way (Tsuyuki et al., 1965) in 0.023 M sodium borate buffer, *p*H 8.5, and containing 0.025% disodium salt of ethylenediaminetetraacetic acid. For the convenience of working under conditions of limited facilities, the degassing step during the preparation of the gels could be omitted by allowing gas bubbles to rise to the surface. Comparative tests on actual hemoglobin separations from many species of fishes showed no basic difference in their patterns by the omission of this step.

The internal dimensions of the mould used for pouring the gel is 7 inches long, $1\frac{7}{16}$ inches wide and $\frac{3}{4}$ inches deep (Fig. 3). The plexiglass end pieces are held in place by sturdy rubber bands. To facilitate removal of the gel block from the mould for slicing purposes, a $\frac{1}{16}$ inch glass plate of the same internal dimensions is placed on the bottom. For subsequent slicing, snugly fitting plexiglass dividers $\frac{1}{16}$ inch thick and 10 inches long are inserted beneath the glass plate. After slicing the rough top surface of the cool gel, additional slices are made by inserting dividers. For these thin slices, Stadie-Riggs tissue slicer blades ground to a width of $\frac{1}{8}$ inch were found to be satisfactory. The sliced gels are removed conveniently by layering a 2 mil thick piece of clear "mylar" of the same width and peeling from one end, usually by using the slicer blade to lift the end. Another similar piece of mylar is used to cover the other surface of the sliced gel. Mylar was found to be an excellent material

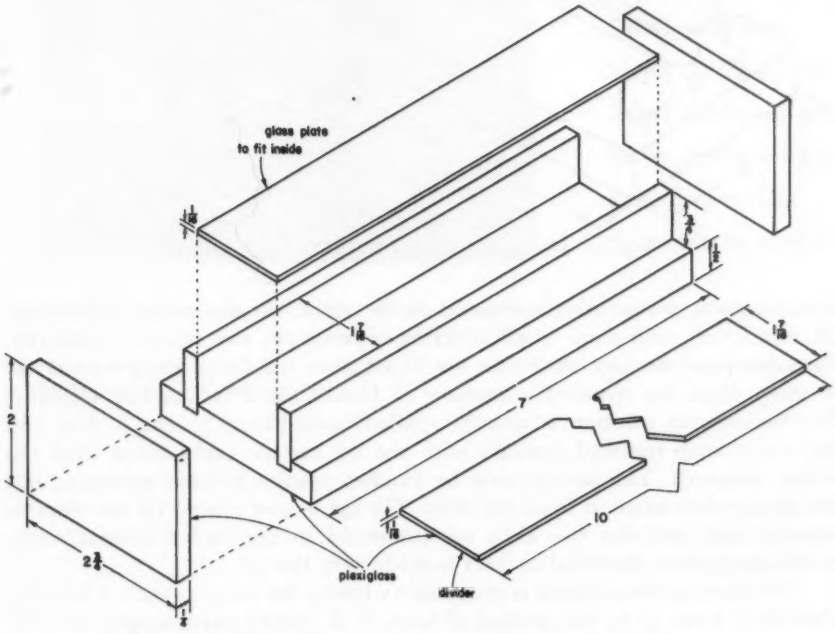

FIG. 3. Starch gel casting mould assembly (dimensions in inches).

for electrophoresis because it has good insulating qualities and does not have a tendency to crinkle and trap air like saran, or roll as many other synthetic films. In this way the handling of the thin delicate gel slice is facilitated and surface evaporation of moisture is effectively prevented.

For the insertion of the sample, a slotter was fashioned from an ordinary Gillette razor holder, the handle end of which is drilled and attached to a stainless steel welding rod mounted on a plexiglass block as shown in Fig. 4. The cutter is made from stainless steel razor blades notched rectangularly in four places so that five equal slots can be cut into each gel of $1\frac{7}{16}$ inches in width. The guard of the razor head is removed to expose the blades and ground so that the cutting edge is just above the bottom of the block with the razor in the down position. To form the slots two blades are inserted

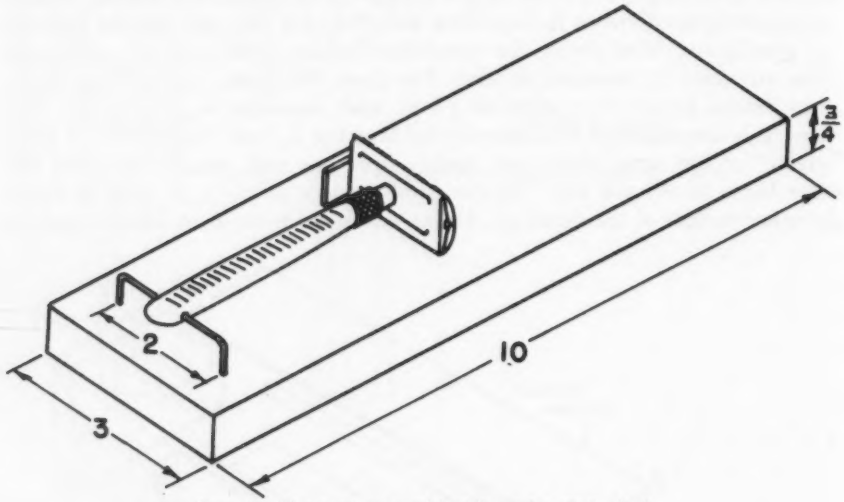

FIG. 4. Slot cutter assembly (dimensions in inches).

into the razor, the gel slice positioned on the block, the top mylar strip peeled off, and a very thin piece of gel adhering between the two blades is removed. For subsequent slotting, the blades are wiped clean and the procedure repeated. If wider slots are required a number of blades could be stacked together. The hemoglobin solution is usually applied immediately to the 5 slots and the mylar strip replaced, contact with the gel surface being made from the center outwards. The gel can now be handled readily without squeezing out the hemoglobin solution from the slots. The gel is now placed on the electrophoresis unit and the two ends are immersed in the buffer compartment. In this way direct electrical contact is made with the gel.

The hemoglobin solution is prepared by lysing the red cells with 2 volumes of distilled water or by the method of Koch et al. (1964) which simply involves admixing the packed cells with a distilled water paste of sephadex G-25 which

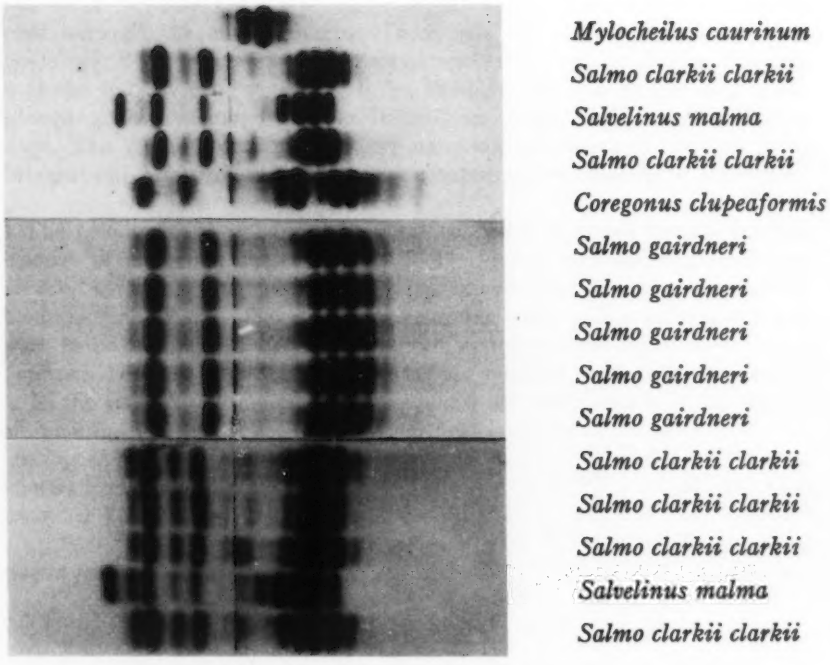

FIG. 5. Starch gel separation of the multiple hemoglobins from a number of fishes (cathode to left, anode to right).

absorbs salts creating an ion-free aqueous medium. After centrifugation the concentrated solutions of hemoglobins are drawn up into capillaries made from 1.3–1.5 × 75 mm microhematocrit tubes, and introduced into the slots either by capillary action or by carefully drawing the tip across the slot.

Electrophoresis is carried out for 2 hr at 160 v. At the completion of the separation the upper mylar strip is removed and the gel placed in a shallow plexiglass staining tray divided into eight sections of internal dimensions 2 × 8½ inches. The dividers in each section are constructed ⅛ inch short of the sides to allow for equilibration of the amido black solution or wash solvent between the compartments. Three gels are placed side by side on pieces of 4⅛ × 7⅝ inch transparent mylar sheets of 5 mil thickness, inserted into Model B-18 Whirl Pak Bags (Hydro Products Division of Oceanographic Engineering Corporation, P.O. Box 10766, San Diego, Calif.) and sealed for storage. The mylar sheets enable easy handling and prevent the gels from disintegrating. The protein patterns are photographed directly in the sealed bags.

The resolutions that have been obtained of hemoglobins from a number of species of fishes are shown in Fig. 5. The degree of resolution compares favorably with that achieved in previous experiments on the same species (Tsuyuki et al., 1965). The extent of reproducibility and species specificity appear to be retained in the micro as well as in the previously used macro techniques. Distinct advantages, however, are realized in the shorter time of run, in the smaller volume of sample required and particularly in its general usefulness for analyses at sea and at other field facilities.

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Southern Occurrence of *Acantholiparis opercularis* Gilbert and Burke in the Eastern Subarctic Pacific Region

The spiny-cheek liparid is a bizarre cold-water species known to inhabit deep waters in and around the Bering Sea region. It is readily diagnosed from other Liparidae by the presence of at least two strong opercular spines and absence of pyloric caeca.

A single male *Acantholiparis opercularis* Gilbert and Burke was caught during the Bureau of Commercial Fisheries–Atomic Energy Commission study, based at Seattle, Wash. The fish was taken in a 43-ft semi-balloon shrimp trawl off the northern Oregon coast (45°40′N lat, 124°55′W long), August 28, 1963, at a 1372 m station, and deposited at the University of Washington (UW 17036) (Fig. 1).

The following descriptive information taken from the new record supplements Gilbert and Burke (1912) and Burke (1930) and contributes new

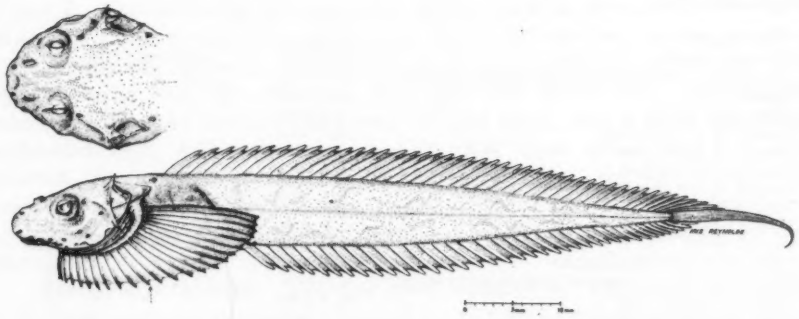

FIG. 1. Illustration of *Acantholiparis opercularis* Gilbert and Burke (UW 17036), showing cephalic sensory pore arrangement and spinate opercle.

data on variation in this species. Counts and measurements were taken from the left side of the specimen whenever possible. Counts were made by dissection when necessary, with the aid of radiographs, and measurements made with dial calipers to the nearest 0.1 mm (both recorded by standards outlined in Hubbs and Lagler, 1958). Total vertebrae count included complete centra plus the urostyle.

Scales absent; body elongate, tapering, and covered with loose skin; head and trunk region ventrally depressed. Fine, conical teeth, recurved distally, present only on upper and lower jaws in 21–25 oblique rows; each row possessing 2–7 uniformly sized teeth; occasional single tooth displaced from banded pattern. Darkly pigmented tubular nostril located closer to eye than tip of snout. Cephalic pore system well defined when specimen is immersed in water (Table I, Fig. 1).

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TABLE I. Comparison of UW 17036 with existing descriptions of *A. opercularis*.

Character	UW 17036	Burke (1930)	Gilbert and Burke (1912)[c]
Meristic data:			
Dorsal rays	45	45	
Anal rays	38	39	
Caudal rays	9 (5 ventral)	10	
Pectoral rays	24	26	
Total vertebrae	50		
Caudal vertebrae	41		
Gill rakers	8		
Gill arches	4		
Branchiostegals	6		
Cephalic pores:			
Prenasal (supraorbital)	1	1	
Infraorbital	6		
Postorbital	1		
Preoperculo-mandibular	5		
Suprabranchial	1	1	
Morphometric data:			
Total length	83.4 mm		
Standard length	69.1 mm	76 mm	
Head length (to posterior edge of gill opening)	15.0 mm	4.6 L[a]	23
Head width	15.8 mm		18
Max. head depth	7.0 mm		13
Length of gill opening	2.9 mm		
Length of upper jaw	7.1 mm		
Length of lower jaw	6.4 mm		
Length of jaw overhang	0.5 mm		
Length of snout	4.9 mm	3 H[b]	7
Interorbital width	2.6 mm		9
Orbital width	3.2 mm	4.8 H	4.5
Width of nostril (horizontal)	1.0 mm		
Max. body depth (taken at mid-pectoral length)	8.0 mm	7 L	14
Length of dorsal base	54.1 mm		
Dorsal overlap on caudal	6.0 mm		
Length of anal base	48.8 mm		
Anal overlap on caudal	3.0 mm		
Length of caudal	14.0 mm	1.2 H	
Length of pectoral:			17
Upper insertion and rays	12.0 mm		
Lower insertion and rays	3.0 mm		
Width of pectoral base	9.7 mm		
Predorsal length	16.4 mm		
Preanal length	23.6 mm		
Snout to pectoral:			
To upper insertion	14.1 mm		
To lower insertion	5.5 mm		
Snout to center of anus	14.1 mm		17
Anal-Anus interspace	9.3 mm		

[a]In standard length.
[b]In head length.
[c]In hundredths of standard length.

Opercle with two distinct dorsally- and laterally-directed spines branching from central focal point mid-length on anterior margin; two ribs radiating along post-ventral portion (Fig. 1). Preopercle sculptured on ventral arm with three enlarged cavernous pores. Subopercle anteriorly originating loosely in skin between preopercle and ceratohyal (at about the origin of the third branchiostegal ray); ending posteriorly at juncture of preopercle with opercle; folding with, but lying above the four outer branchiostegal rays. No slit behind the fourth gill arch; pseudobranchiae and thymus absent. Gill rakers rudimentary blunt knobs on lower arm with distal spinous projections. Origin of dorsal above a point about mid-length of pectoral fin (measured from lower insertion); anal fin beginning beneath seventh dorsal ray. Caudal fin well-developed and long; last dorsal ray overlying not quite half the caudal fin length; last anal ray overlying less than one-quarter its length. Pectoral rays shortened anteriorly and evenly spaced. Anus positioned well in advance of anal fin about mid-pectoral length. Posterior one-eighth of intestine enlarged to nearly twice the diameter of the anterior portion. Testes bilobed; positioned on either side of urinary duct and occupying posterior one-third of coelomic cavity. Head and body sparsely pigmented except for black on lips, fin emargination, and abdominal region. Mouth, branchial chamber, peritoneum, intestine, and stomach all dusky. Remainder of description presented in Table I, in Gilbert and Burke (1912), and in Burke (1930).

Variation. The new record exhibited little meristic and morphometric variation from the type-description; the former with fewer pectoral fin rays, slightly more slender body, wider head, and narrower interorbital width (Table I). The difference in interorbital width could have resulted from measuring UW 17036 across the frontal bones, not to include the extraneous tissue. By a comparison between illustrations of the type (Gilbert and Burke 1912: fig. 28; Burke 1930: fig. 104) and the larger new record (Fig. 1), it appears that the latter has a more completely formed snout, and a more posteriorly positioned vent. Although it is probable that these differences may have resulted from the poor condition of the specimen(s), the effect of growth must not be precluded. The new record varied from the type-description by less than 4% of the standard length in all other morphometric features mentioned.

Records of occurrence. Acantholiparis opercularis was formerly known from three specimens initially recorded by Gilbert and Burke (1912) ranging from off the southeastern tip of Kamchatka (52°38′N lat, 158°50′E long) in 1247 m westward to below the Gulf of Alaska, near the Shumagin Islands (53°58′N lat, 159°31′W long) in 3609 m; the range is now extended south by a fourth specimen from off the northern Oregon coast (1372 m).

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Characteristics of Scales from Pink Salmon (*Oncorhynchus gorbuscha*) Transplanted from the Glendale River in Central British Columbia to the North Harbour River in Newfoundland

In 1962, pink salmon eggs from the Glendale River situated in the central coastal area of British Columbia were transferred to spawning beds in the North Harbour River in Newfoundland, to which some progeny returned to spawn in 1964. Scales from seven of these spawning fish were obtained for study. Using a microprojector at 100 magnifications, for each scale, the number of circuli in the first year (from the center of the scale focus to the outside edge of the last circulus of the annual ring) were counted and the average space between circuli in the first year band was computed.

In Table I, measurements for the transplanted pinks are compared with those of the native population for the same year in the parent Glendale River and with other Pacific populations of the same year from the Bella Coola River in British Columbia northward to the Alaska Peninsula. The transplanted pinks had significantly fewer and more widely spaced circuli than did their siblings in the Glendale River (number circuli, $t = 4.6874$,

TABLE I. The average number of circuli and the average space between circuli in the first year for scales of pink salmon sampled from various locations along the North Pacific coast of America in 1964, and for the scales of pink salmon which returned to Newfoundland in 1964.

Area	Average number first year circuli	Average space between circuli in first year	Total samples
		(*mm*)	
Glendale River (donor stream)	27.73	0.0340	81
Bella Coola River	27.61	0.0325	100
Skeena River	24.86	0.0333	100
Southeastern Alaska	26.27	0.0368	478
Prince William Sound, Alaska	25.03	0.0385	100
Cook Inlet, Alaska	22.85	0.0423	219
Kodiak, Alaska	22.56	0.0409	25
Alaska Peninsula	21.68	0.0414	376
Newfoundland (transplants)	23.14	0.0381	7

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$P = < 0.001$; space between circuli, $t = 2.8320$, $P = < 0.010$). Typical scales from the Glendale River and the Newfoundland fish are shown, respectively, in Fig. 1 and 2. The "transplant scales" were much more similar to those of pinks from central Alaska. These results indicate that the number and spacing of circuli in the first year of pinks is not a fixed genetic character but can be altered when the fish are reared in a different environment.

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(Fig. 1 and 2 *opposite*)

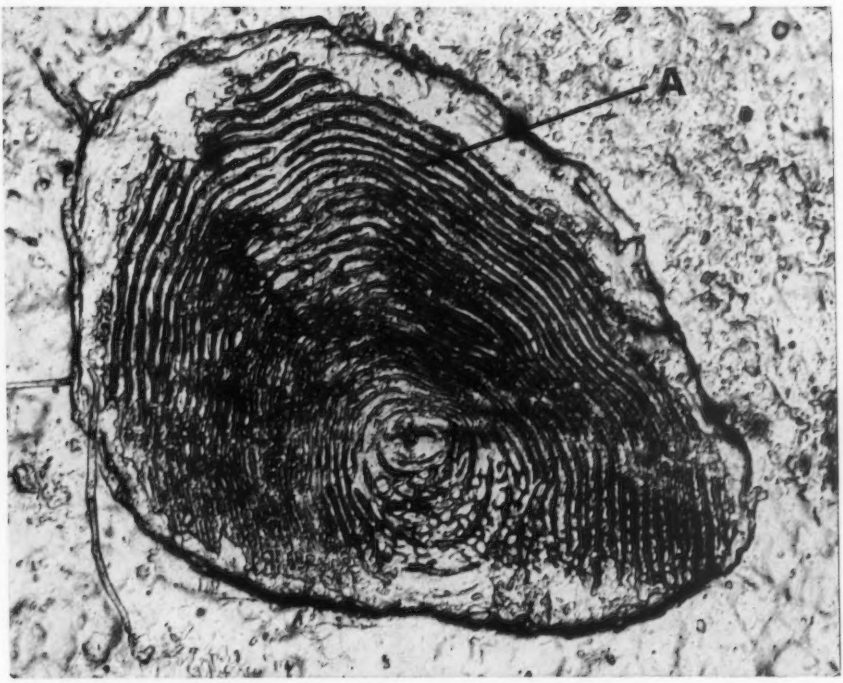

FIG. 1. A scale from a transplanted pink salmon which returned to Newfoundland in 1964, showing the first-year band (A). Note: There were 23 circuli in the first year.

FIG. 2. A scale from a pink salmon which returned to the Glendale River in the central coastal area of British Columbia in 1964, showing the first-year band (A). Note: There were 30 circuli in the first year.

Feeding Relationship of Walleye and Smallmouth Bass

The introduction of exotic fish into an established community may result in interspecific competition for food. The feeding habits of walleye, *Stizostedion vitreum vitreum* (Mitchill), and smallmouth bass, *Micropterus dolomieui* Lacépède, in Falcon Lake, Manitoba, were investigated to test this premise. The walleye is the principal native game species. Smallmouth bass is an introduced species and the population derives from plantings made in 1946. As this population expanded the bass localized in areas along the north and south shores and is now among the most abundant game fishes in the lake.

Walleye and bass samples were collected in these localization areas. The fish were obtained from gillnet and anglers' catches. Their size was: range (FL) smallmouth bass, 10–18 inches, walleye, 13–28 inches. Nearly 75% of the bass were between 13 and 15 inches and about 60% of the walleye were between 12 and 16 inches long.

TABLE I. Occurrence of food items in stomachs of 288 walleye and 177 smallmouth bass from Falcon Lake, 1959.

Food item	Percentage frequency		Intensity			
			Average[a]		Relative[b]	
	Walleye	Bass	Walleye	Bass	Walleye	Bass
Fish	58	49	1.1	0.6	1.8	1.2
Catostomus commersoni	4	0	0.1	0.0	1.1	0.0
Cottus sp.	0.4	0	0.1	0.0	1.0	0.0
Cyprinids	2	0	0.1	0.0	1.0	0.0
Leucichthyids	0	1.3	0.0	0.1	0.0	1.0
Micropterus dolomieui	2.7	0	0.1	0.0	1.3	0.0
Perca flavescens	16	16	0.5	0.2	2.7	1.3
Pungitius pungitius	0.7	2	0.1	0.1	1.5	1.3
Stizostedion vitreum vitreum	4	0	0.1	0.0	1.6	0.0
Unidentified	30	28	0.5	0.3	–	–
Crayfish	3	68	0.1	1.2	1.7	1.7
Amphipods	9	0	0.2	0.0	2.0	0.0
Insects	94	77	18.8	2.1	19.5	2.7
Aquatic	86	65	18.6	1.5	21.2	2.3
Ephemerid nymphs	76	21	18.2	2.4	23.5	2.4
Terrestrial and unidentified	8	7	0.2	0.1	–	–

[a]Average intensity = average number of items per fish in total sample.
[b]Relative intensity = average number of items per fish containing that item.

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Over the sampling period, stomach contents of 234 bass and 384 walleye were examined. Stomachs were removed soon after capture, partially slit, wrapped in gauze, and preserved in formaldehyde. In the laboratory, the contents were teased out under water, examined and sorted into taxonomic groups. Individual organisms in each group were identified and counted.

The feeding habits and subsequent feeding relationship of bass and walleye were determined by analysing: (1) the kinds of food items taken by each fish; (2) the number of specific items taken by each fish; (3) the seasonal occurrence of items in fish stomachs.

About 25% of both walleye and bass stomachs were empty; these were entirely free of food remnants or contained small amounts of unrecognizable detritus. Insects, fishes, and crustaceans comprised the contents of the remaining 288 walleye and 171 smallmouth bass stomachs. The frequency of occurrence of these items, as summarized in Table I, relates only to the stomachs with food contents.

Crayfish (*Cambarus* sp.), found in 68% of the bass stomachs (Table I), was the major food item of bass. Insects were eaten with comparable frequency but because few were taken at any time and were small in size they provided insufficient bulk to rank them as important as crayfish. The insect item was made up chiefly of aquatic forms among which ephemerid nymphs occurred most frequently, but rarely more than four or five were found in any one bass stomach. The significant fish item for bass was yellow perch (*Perca flavescens*); it occurred in 16% of the stomachs but with an average intensity of 0.2 compared to the degree, 1.14, which crayfish were attained (Table I).

Walleye rarely ate crayfish. They fed most frequently on ephemerid nymphs which occurred in 76% of the stomachs. As many as 70 nymphs were found in a single stomach but they occurred with an average intensity of about

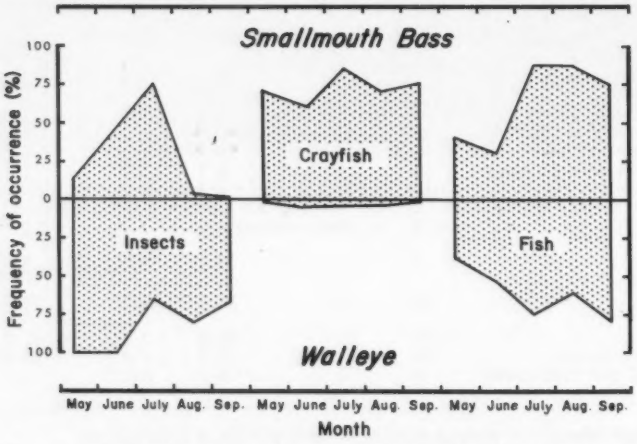

FIG. 1. Monthly frequency of occurrence of the three food categories in the stomachs of smallmouth bass and walleye, Falcon Lake, 1959.

24 per fish. Few other insects were eaten. Fish items, occurring in 58% of the stomachs, were of next importance to walleye. Yellow perch was the most frequent specific fish item; it occurred as often as it did in bass stomachs. Walleye, however, ate perch two or three times more intensively than bass (Table I). Included among the other fish eaten were both young walleye and smallmouth bass. Bass, on the other hand, were neither predacious on walleye nor cannibalistic.

Bass fed on crayfish with nearly the same frequency and intensity over the sampling period (Fig. 1). In early May this item coupled with fish and a few insects made up their diet. Bass ate increasingly more insects over late May through June to mid July, at which time they took them at maximum frequency. Subsequently, they took progressively fewer insects, almost foresaking them altogether as food by the end of August. Their feeding on fish increased steadily from early June to late July when the maximum level was attained, and more or less remained constant through the rest of the study period. Over the latter months the bass diet consisted almost totally of crayfish and fish.

Ephemerid nymphs occurred in nearly every walleye stomach until mid June whence the frequency decreased steadily to about 67% by mid September. This decrease was offset by an increase in the number of fish eaten. Walleye began this more extensive feeding on fish in late June.

These observations indicate that smallmouth bass have moved into the Falcon Lake complex without initiating a competitive food interaction with walleye. The species ate a number of common food items, but the pattern of feeding intensity demonstrated that each fish had peculiar preferences. Over the sampling period, bass were sustained by crayfish and walleye by ephemerid nymphs. Fish items were of secondary importance in bass diets; walleye, however, ate a wider variety than bass, thus minimizing competition.

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